A portion of this document (Attachment 11, Pages 4-8 and Attachment 19, Pages 10-13) have been claimed confidential. It is releasable to persons who submit a signed "Affirmation of Non-Multinational Status" form.

ATTACHMENT 1

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

November 26, 1997

Permit me to compliment you and the Cancer Assessment Review Committee (CARC) on the fine work and general decision rendering process pursued at the September/October assessment of the malathion carcinogenicity data base. I was particularly pleased over the invitation extended to Richard Brown to participitate at the meetings in the capacity of facilitator. Richard was very helpful to me in re-ordering some of the information for presentation to the committee as well as in rendering advice on how to be a presentor/commentor.

The Committee's decisions to require additional histopathologic assessments of various tissues, as enunciated in the November 3 memorandum of Jess Rowland was in all cases entirely appropriate. Also it was very encouraging to me to find in Jess' memorandum the publication by Eldridge, et al (1995) depicting the appropriate techniques for histopathologic assessment of nasal tissues.

Having expressed these views, and not knowing what the future holds with respect to my continued involvement with malathion, particularly at such time as when those final deliberations are held on the carcinogenicity of malathion, I consider it imperative to introduce into the record (as if I were to no longer be involved) certain specific follow-up views on the recent CARC.

I find unacceptable the notion that cholinesterase inhibition in a chronic carcinogenicity study should be used to conclude that dosing was excessive in the absence of clinical signs, increased mortality, substantial deficits of body weight gain or other evidence an MTD was exceeded. In all of my extensive involvement with the cholinesterase project I have witnessed nothing that reveals a relationship between cholinesterase inhibition and carcinogenicity when animals were not exhibiting clinical signs or increased mortality. Cholinesterase is assayed in the case of organophosphates because these are cholinesterase inhibitors and the LOEL/NOEL is needed to address cholinergic toxicity. Other enzymes, possibly more related to carcinogenicity such as DNA-repair enzymes, adenyl cyclase, glycolytic enzymes, plus a host of others are not assayed. If any of these were remarkably inhibited, would we conclude as in the case of cholinesterase inhibition that dosing is excessive, and discount tumor findings at those doses? Mechanisms of carcinogenesis are not understood, and due in part to this deficiency of understanding, high dose testing is pursued. It is well recognized that such doses likely far exceed those levels people would be exposed to for any length of time, and would be anticipated to alter many enzyme systems, cholinesterase inhibition not withstanding. It could be argued that there is a selective testing advantage for cholinesterase inhibitors over other classes of chemicals (pesticides included) that don't suffer this compromise in reaching high doses because of interfering cholinesterase inhibition. Further, it could be argued that to properly test the more potent cholinesterase inhibitors, cholinesterase inhibition needs to be circumvented to get to those higher doses as is done in the case of testing for delayed neuropathy (OPIDN) through the use of atropine. In the case of the recent malathion mouse carcinogenicity study (MRID 43407201) this was in effect achieved as the animals survived high doses, without evidence an MTD was exceeded. In essence, my point is that cholinesterase inhibition should not

have been used in the case of the malathion mouse carcinogenicity study to discount the very remarkable tumorigenic responses in mice of both sexes at the two high dose levels. In support of this I would quote from EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment:

> "Animal studies are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity of the tested agent. There is little doubt that this may happen in some cases, but skepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al, 1993a; Melnick et al, 1993b; Barrett, 1993). In light of this question, the default assumption is that effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized. If adequate data demonstrate that the effects are solely (emphasis added) the result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent." (p. 27)

Now in view of all of these considerations, I am not aware that anyone demonstrated at the CARC, either on the rationale of cholinesterase inhibition or any other parameter of toxicity, that the tumorigenic findings in the mouse study were "solely the result of excessive toxicity rather than carcinogenicity of the tested agent per se". Indeed the remarkable tumorigenic findings in male mice at the lowest dose (100 ppm) would dispute such a conclusion, barring a change of mechanism.

As to the question of the limit dose being exceeded in the mouse study (dosage levels: 0, 100, 800, 8000 or 16000 ppm), this is clearly marginal at 8000 ppm and not sufficiently exceeded at 16000 ppm to merit discounting the findings at these doses. I say this in light of the fact that under the FIFRA Subdivision F Guidlines for carcinogenicity testing the limit dose is 5% of the diet, or 50,000 ppm. Only in more recent times has it been revised by internal memorandum to 7000 ppm for mice. If one were designing a study today perhaps the highest concentration of malathion in the food to be tested would be 7000 ppm, but now that the study has been conducted at 8000 and 16000 ppm as required of the registrant specifically to address so called questionable findings in the earlier National Cancer Institute study, I find it incredible that people would elect to discount positive findings at these doses in the current study if they were seriously interested in providing public assurance of the minimal risk. Furthermore, in my judgement, cholinesterase inhibition alone does not satisfy as sufficient reason to discount these findings. From the perspective of public health considerations, a much more compelling argument must be presented before the liver tumor findings are to be discounted. But if the Committee insists upon discounting findings at these two dose levels, there is an encumbency to test at 7000 ppm, viewed as the limit dose for mice. To permit the next lower dose below 8000 ppm, namely 800 ppm, to serve as an adequate high dose for the current study is to deny proper testing in the mouse for carcinogenicity of malathion.

I find it unfortunate to have to express these views after the CARC meetings, but quite frankly I was surprised at the invoking of cholinesterase inhibition as a way of discounting tumorigenic findings

and needed additional time to reflect on the issue. At such time as this matter is revisited after the Pathology Working Group has rendered an opinion on the mouse liver tumors, I must put the challenge to CARC members to produce reasonable evidence to substantiate that cholinesterase inhibition, in the absence of other evidence of excessive toxicity, was somehow responsible solely, or even primarily (walking that extra mile), for the tumorigenic findings in the malathion mouse study. **I recommend this question to the Science Advisory Panel, if not previously addressed by that body, the question being the appropriateness of using cholinesterase inhibition in the absence of any other evidence an MTD was exceeded, to discount tumor findings.**

Another matter of considerable importance is that of nasal tissue lesions identified in the new malathion chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). As I recall, when the Committee engaged this topic, there was little, or inadequate, discussion of the tumorigenic findings. Rather, the Committee quickly acknowledged that all nasal tissues had either not been examined or not fully examined, and elected to call in the additional histopathology assessments, deferring until such data is received a decision on this tumorigenic endpoint. To the extent that malathion may continue to be used during this interim period, I consider it my responsibility to advise the Committee of certain findings that exist in the data base. Firstly, the nasal tumors identified in this study, an adenoma among males in the 6000 ppm group and a carcinoma among males of the 12000 ppm group were described in the MRID study report itself as rare compound related tumorigenic findings. However, as explained in the DER of the study, while the tumors were characterized in the MRID study report as rare: "Spontaneous neoplasms of the nasoturbinal tissues are rare in F344 rats. In untreated dietary and corn oil control animals from eight recent NTP studies only six were identified from nearly 4000 control males and none occurred in a similar number of control females (citing Boorman et al, 1990). None have been observed in this laboratory in six previous studies (238 control males and 241 control females." (P. 93 of MRID study report) As explained in the DER (p. 62), both nasal tumors identified in the study were of the olfactory region of the nasal mucosa. An independent reading of Boorman et al (1990) confirms nasal tumors as rare among NTP historical controls, but just how rare was understated in the MRID study report. As written in the DER, "However, the claim of some six tumors among nearly 4000 control males is with reference to the respiratory epithelium (confirmed by personal communication with the principal author and inspection of Haseman et al (1990). Boorman et al (1990) and Haseman et al (1990) claim/identify **zero** incidence of tumors of the olfactory epithelium from among nearly 4000 control male rats, and none among a similar number of control females. In fact, Boorman et al (1990) says 'Neoplasms of the olfactory epithelium have occurred in F344 rats exposed to certain carcinogens, but have not been observed in controls.' (P. 332) So the finding in this study of two such tumors of the olfactory epithelium is exceedingly rare indeed, and heretofore unique to carcinogens." (p. 62 of DER)

Given the above, it is important to recognize that in addition to the rare tumors, hyperplasia and other non-neoplastic lesions of the olfactory epithelium were observed with high incidence in rats of both sexes at both 6000 and 12000 ppm. However, these lesions were not increased at or below the 500 ppm dosing level in the chronic toxicity/carcinogenicity study after a full two years. To the extent that hyperplasia was a precursor event to tumorgenic findings of the olfactory epithelium in this study (which we cannot actually say in this case), the fact that hyperplasia of the olfactory epithelium was not observed at or below 500 ppm is of some encouragement that such tumors may not be expected

at those doses.

The added concern I have and wish to make note of is that the subchronic inhalation study on malathion (MRID 43266601) revealed hyperplasia of the olfactory epithelium in nearly all animals, both sexes and all dose levels. There was no NOEL, where concentrations employed were 0.1, 0.45 and 2.01 mg/liter. These concentrations when expressed in dosages delivered to the animal as calculated from inhalation concentrations using the best in-house procedure (mathematical formula) available for obtaining such estimates, were 4.7, 21.2 and 94.5 mg/kg/day, or in terms of ppm in the diet, 75, 340 and 1508 ppm, respectively. These are very gross estimates with several qualifiers, as related to me by one of HED's inhalation experts who provided the formula. However, in terms of comparative inhibitions of erythrocyte cholinesterase, inhibitions among female rats in the chronic toxicity/carcinogenicity study after three months were 24% and 30% at 100 and 500 ppm, as compared to 11% and 27%, respectively, at the estimated doses of 75 and 340 ppm in the inhalation study at the same time point, three months. There is fair agreement in these findings in the two studies, suggesting that the estimated dosages delivered in the inhalation study were not far removed from those by the oral route. Thus after only 90 days of treatment, hyperplasia in the inhalation study extended to a much lower dose, estimated to be equivalent to that of 75 ppm in the diet (without a NOEL), than in the oral feeding study where the NOEL/LOEL was 500/6000 ppm. This may not be surprising given the direct application of the agent to vulnerable nasal tissues via the inhalational route. We have no idea how much earlier than 90 days of treatment hyperplasia might have occurred.

Since there was no NOEL in the inhalation study, exposures to low and possibly unknown concentrations by this route are problematic in terms of affirming public safety via the inhalational route of exposure. In my opinion, there is the need for additional review of this topic during the interim period that malathion continues to be used while the nasal tissue effects are being evaluated.

Also, it is my recommendation that when the CARC convenes again to consider the carcinogenicity of malathion, that you re-visit the mononuclear cell leukemia and interstitial cell testicular tumor data. I am concerned that these findings were dismissed too quickly by the Committee at the September/October meetings. In my judgement, in both cases competing toxicity, excessive early mortality and causes of death as matters pertaining to the proper selection of statistical methods of analysis and interpretation were not given adequate attention.

In closing, I would again compliment you and the Cancer Assessment Review Committee for your fine work in evaluating the malathion carcinogenicity data base.

Brian Dementi, Ph.D.
Toxicologist

cc Jess Rowland
Richard Brown

ATTACHMENT 2

To: Bill Burnam February 23, 1998

From: Brian Dementi

Re: Comments on your draft note to Judy Hauswirth/JSC regarding the meeting of February 18 in your office.

The following summarizes my understanding of the items of interest at the said meeting:

The PWG was intended for male mouse liver. I offered the views of NTP's Dr. Robert Maronpot who advised me that equal amounts of tissue from each mouse should be examined, and this should come from slides of at least three lobes of the liver. After our meeting on 2/18, I reexamined the mouse study report and found that for liver histopathology: "2 lobes examined; 3 sections collected" (p. 18) . I spoke with Dr. Maronpot who expressed the view that since three sections were examined from each of two lobes, this should be satisfactory for the PWG to consider. I should note that all gross lesions, regardless of the lobe, are also included in the microscopic assessment. So with respect to item 1 of your memo, the bottom line is we all agree that the slides currently available, assuming equal amounts of tissue are represented from each mouse, are suitable for the PWG assessment.

Item 2 of your memo is consistent with my understanding, but requires some embellishment. Specifically, advise that inspection of nasal tissue slides should include careful examination of the squamous epithelium lining the alveoli of roots of teeth, where two rare tumors (squamous cell carcinoma) have been identified so far in dosed groups of this study. Concerning the uterus, Dr. Maronpot advised and Dr. Brennecke agreed that three sections be examined, one from each uterine horn plus one from the cervix of each rat. Concerning the pituitary, both of these pathologists agreed that the critical section for examination is one through the widest region of the pituitary such that both lobes are represented.

Item 3 of your proposed response is consistent with my understanding.

Please submit additional statistical analyses of tumor data from the malaoxon chronic toxicity/carcinogenicity study in rats (MRID 43975201). Specifically, the additional work should be directed to mononuclear cell leukemia, both sexes, and interstitial cell testicular tumors in males.

You will recall I read the group portions of a July 24, 1997 letter from Dr. Maronpot addressed to me regarding the PWG, wherein he recommended that "It would also be beneficial to have yourself or another EPA toxicologist participate in the peer review process as an observer to insure that all important questions you might have are resolved." Dr. Brennecke appeared to agree with this recommendation. Given my long history with the malathion toxicology data base, and having presented at both cancer peer reviews (1990, 1997), I believe I should fulfill that role of EPA toxicologist on the PWG as indicated.

ATTACHMENT 3

TO: Mike Ioannou
Bill Burnam

April 9, 1998

FROM: Brian Dementi

Mr. Paul Whatling of Jellinek, Schwartz & Connolly forwarded to me under a confidential letter dated April 3, 1998 a copy of an April 3, 1998 hand delivered letter to Ms Dana Lateulere, Reregistration Branch, setting forth a schedule and detailed summary of work to be completed on the additional evaluations of tissues and slides from the malathion mouse carcinogenicity and chronic toxicity/carcinogenicity study in the rat. I have read this letter and the objectives are in accord with my understanding of what was requested of the registrant.

On page 2 of the letter concerning the Pathology Working Group (PWG) Review on male mouse liver tumors, scheduled for April 28 and 29, 1998, acknowledgement is made of the Agency's indication that it may be beneficial to have an EPA toxicologist participate as an observer, and offers to try to arrange for this participation. As you know, Dr. Robert Maronpot of the National Toxicology Program, who has participated in many PWGs, had suggested the involvment of a toxicologst in the PWG meeting in his letter to me of July 24, 1997: "It would also be beneficial to have yourself or another EPA toxicologist participate in the peer review process as an observer to insure all important questions you might have are resolved." A copy of his letter is attached.

As I indicated at a meeting we had to develop a statement of what should be expected in the assessment of these studies, I would like to serve as toxicologist observer on the PWG. I am interested in this particular chemical having worked on it for years, and believe the opportunity to witness the process would be of value to me professionally. Could I expect that someone within HED would authorize my attendance and so inform the registrant, or is this arragement in my court? I would hope to hear something as soon as possible, as I plan to be on vacation next week.

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. 0. Box 12233
Research Triangle Park, NC 27709

24
July ~~14~~, 1997

Dr. Brian Dementi
U.S. Environmental Protection Agency
Mail Code 7509C
401 M Street SW
Washington, DC 29460

Dear Dr. Dementi:

I have reviewed the material you provided to Dr. Haseman and his letter to you dated July 17,1997. I concur with all of Dr. Haseman's suggestions and opinions. Since the liver tumor response across the various dose groups is unusual, a formal peer review of the histopathological diagnoses is warranted. In the absence of such a peer review, the present findings would indicate a clear liver tumor response at the 100, 8000, and 16000 ppm levels. Should all of the original diagnoses be confirmed, you could proceed with more certainty in arriving at a judgment regarding the outcome of this study. Because of the unusual tumor incidence findings it is imperative that any peer review be carried out without knowledge of the treatment status for each mouse. I recommend that all liver slides from all mice be subjected to peer review and that particular attention be paid to insuring that all grossly observed liver nodules have been appropriately made into histologic slides. Furthermore, it is imperative that the peer review insure that equivalent amounts of liver tissue have been made into histologic sections from all mice. Thus, all gross liver lesions should have a corresponding histologic diagnosis and equivalent amounts of grossly normal liver should have been processed into histologic slides.

A number of private pathology organizations have experience in conducting pathology peer reviews and I can provide names and addresses

should you desire. I am willing to offer the participation of our National Toxicology Program senior pathologists in the peer review exercise. It is also wise to include the original study pathologist in the process and a qualified pathologist from academia. It would also be beneficial to have yourself or another EPA toxicologist participate in the peer review process as an observer to insure that all important questions you might have are resolved. Again, I stress the importance of reviewing all liver tissues, even from animals without diagnosed liver tumors, since additional neoplasms may be found and preneoplastic lesions of the liver may be documented in some mice without overt liver tumors.

Sincerely yours,
/S/

R. R. Maronpot, DVM
Chief, Laboratory of Experimental Pathology

ATTACHMENT 4

Jerry Hardisty, D.V.M.
Experimental Pathology Laboratories, Inc
P.O. Box 12766
Research Triangle Park, NC 27709

May 4, 1998

Dear Dr. Hardisty,

As a follow-up to the Pathology Working Group (PWG) convened April 28-29, I have a few comments.

Having now had the opportunity to read the 1980 publication you provided by Dr. Ward on "Morphology of Hepatocellular Neoplasms in B6C3F1 Mice", I find it significant that the article indicates that the size of a liver tumor appears to bear a positive relationship with the likelihood that trabecular formations are present. Accordingly, the paper says the following: "Small tumors, usually 1-5 mm in diameter, were most commonly composed of a uniform population of basophilic hepatocytes growing in a solid pattern, with a cell size smaller than normal hepatocytes (Fig. 1). Other small nodules contained predominantly eosinophilic or vacuolated hepatocytes, or a mixture of all 3 cytoplasmic types. The eosinophilic and vacuolated cells were generally larger than normal hepatocytes. The uniform population of hepatocytes and the general difficulty in transplanting these tumors [5,8,19] led to their diagnoses as hepatocellular adenomas." (p. 321) Further along the paper says: " The large liver tumors [5-10 mm] frequently resemble the small tumors histologically, but also frequently had foci of vacuolated (glycogen or fat) cells, intracytoplasmic inclusions and areas of prominent trabecular formations (Fig. 2), the inclusions were of the Type 2 previously reported [6]. The morphology of hepatocytes in trabecular areas found in 53-55% of the mouse liver tumors were identical to those found in trabecular carcinomas. These trabecular foci in adenomas have been previously reported in mice [3,4,11,15] and may represent the early stages of trabecular carcinoma. The presence of these foci should lead to diagnosis as carcinomas(emphasis added). The largest tumors (greater than 1 cm in diameter) were generally composed of a variety of areas; some resembling adenomas and other larger areas of prominent trabecular formations (Fig. 3)." (pp. 321-323) "The small tumors were composed primarily of basophilic hepatocytes which grew in a solid adenomatous pattern. Large solid tumors had foci of prominent trabecular formations." (p. 319)

The reason for citing this information from Dr. Ward's publication rests with the fact that with respect to hepatocellular adenomas and carcinomas, eleven of the thirteen tumors identified macroscopically in Group 2 (100 ppm) appear to be much larger than the four identified in Group 1 (0 ppm) in the study being considered by the PWG. Given my uncertainty as to just what information was available to the committee, I have decided you should be advised of the size disparity. Accordingly, tumor sizes for Groups 1 and 2 as provided on individual animal pathology sheets are reproduced as follows by animal identification number (note in certain instances dimensions were given in cm which I converted to mm):

Group I

#48885: "nodule", 6 mm diameter
#48897: "nodule" 2 to 6 mm diameter
#48916: "nodule", 5 mm diameter
#48919: "nodule", 4 mm diameter

Group 2

#48994: "nodule", 5 mm diameter
#48995: "nodule", 6 mm diameter
#49006: "mass", 12 x 8 x 6 mm
#49012: "mass", 15 x 10 x 10 mm
#49018: "mass", 8 x 8 x 6 mm
#49019: "mass"A, 7 x 9 x 4 mm
"mass"B, 6 x 7 x 5 mm
#49020: "mass"A, 25 x 20 x 20 mm
"mass"B, 15 x 10 x 10 mm
#49025: "mass", 28 x 16 x 12 mm
#49026: "mass", 20 x 12 x 5 mm
#49052: "mass"A, 15 mm diameter
"mass"B, 10 mm diameter

As you can see, lesions from all four Group 1 mice are described as "nodules" having sizes approximating those of the upper end of the range for small tumors (1-5 mm) as characterized in Dr. Ward's paper. In Group 2 mice, there are ten animals with lesions. Three of these have lesions on two lobes of the liver yielding a total of 13 macroscopic lesions. Two of the lesions are described as nodules having sizes similar to those in Group 1, however eleven of the lesions are described as "masses" rather than nodules, and depending upon the formula one uses to compute relative volumes of these lesions, those described as masses exceed the volume of a sphere of 5 mm diameter and six to eight of these exceed the volume of a sphere having a 10 mm diameter. Hence, all of the masses appear to qualify as large lesions with six to eight falling into the largest category as defined in Dr. Ward's paper.

According to Dr. Ward's paper, given that large tumors are likely to harbor foci characteristic of that of the carcinoma classification and given that four carcinomas have already been identified in Group 2 mouse livers, it would appear appropriate to examine several slides from each of the "masses" in Group 2 in order to be satisfied with the diagnosis as to tumor type. So a principal question I would have is that of whether a sufficient number of sections through these larger lesions were available to the PWG in order to rule out the presence of localized regions of carcinoma within them, in all cases?

Interpretatively, as to the question of whether Group 2 lesions are spontaneous in character, how much significance should be ascribed to the fact that all four macroscopic lesions in Group 1 are described as "nodules", whereas in Group 2, two are said to be "nodules" while eleven are described as "masses"? Historically, are such masses common among control mice in 18-month, or even 24-month B6C3F1 mouse studies? Does the largeness of these masses suggest earlier onset, i.e. decreased tumor latency? Should any significance be ascribed to the presence of three cases in Group

2 of lesions on two lobes of the liver (note also the lesion in #49025 is described as attached to two lobes), particularly in an 18 month study?

Another point I would mention is that, given the weak historical control data base for 18-month studies, as was discussed at the meeting, how much confidence can be placed in that data base. I should note that among the five studies recorded for the performing laboratory, the incidence of carcinoma was 0 in three of the studies, with the present study contributing yet a forth 0 incidence of carcinoma. Among the remaining two historical studies, one carcinoma occurred in one and three carcinomas occurred in the other from among 50 animals in each group. Suppose these four historical carcinoma slides were on the table at the PWG, do we have any sense as to whether the classifications would have survived the re-examination? Since this historical data base is so small and yet so important, should these historical controls also be examined by the PWG members for purposes of uniformity of interpretation?

Permit me to reiterate that I am not aware of just what information was available to the committee, but having now read Dr. Ward's paper and in view of my uncertainty, I felt it appropriate to advise you concerning the macroscopic findings, which you might consider in addressing the question of whether the tumorigenic findings in Group 2 should be characterized as spontaneous in nature. At your request, the macroscopic pathology for all groups would be available.

I felt that your committee made a very conscientious effort to interpret this study, and having worked more closely with you, personally, at the meeting, I was impressed by your resolve to find consensus on the interpretations of slides. You were also very helpful in explaining things to the observers. All members of the PWG group were sources of insight and enjoyment.

Best Wishes,

Brian Dementi, Ph.D.
Office of Pesticide Programs
Environmental Protection Agency
Washington DC 20460
Mail Code 7509C

ATTACHMENT 5

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

May 29, 1998

The following views I would offer for consideration by the Carcinogen Assessment Review Committee (CARC) as to the interpretation of the malathion mouse carcinogenicity study (MRID 43407201) that was the subject of review by a Pathology Working Group (PWG), convened April 27-28, 1998. As a prelude to my specific comments on that study and its conclusions, I would like to mention two operating principles of carcinogen assessment of particular relevance in this case. Firstly, the Office of Science and Technology Assessment (OSTP) 1985 [Fed. Reg. Vol. 50, No. 50, March 15, 1985, 10372-10442] defines a carcinogen as follows: "A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen." (pp. 10414-10415). A decreased time to tumor is often referred to as decreased latency. Secondly, the mechanism of carcinogenicity for a given chemical may not necessarily be the same across all doses. Any *a priori* assumption that but one mechanism operates at all doses for a given chemical, particularly across a wide dose range, must be questioned. I have no reference readily at hand in support of this assertion, but I have read it and heard it said at cancer assessment symposia. Furthermore, it is fundamentally self evident.

The dose levels in this study were 0, 100, 800, 8000 and 16000 ppm. There is clearly an hepatocellular response in male mice at 8000 and 16000 ppm. I should remind the Committee that doses of 8000 and 16000 ppm were required by the Agency in an effort to resolve so called equivocal (marginally statistically significant) liver tumors in males in the 1978 National Cancer Institute study. In that 1978 study, there was no increase in liver tumors among female mice. It is noteworthy and somewhat surprising that in the recent study, the hepatocellular tumorigenic response (adenomas) in males was far in excess of the increased incidences in the 1978 study; and in females, a remarkably high incidence of liver tumors (84%) was seen in the high dose (16000 ppm) group as compared to that in the female control group (2%). The liver is clearly a target organ, particularly in males and most remarkable in the recent study. A principal question of concern is whether a tumorigenic effect is evident in the low dose (100 ppm) male group (Group 2).

The views I wish to express here have to do with the interpretation of unusual findings in that lowest dose (100 ppm) group. Along these lines I have submitted certain questions in the form of a letter to Dr. Jerry Hardisty, Chairman of the PWG. A copy of that letter is appended. My specific concerns are further developed as follows.

The stated purpose of the PWG was "To determine the incidence of hepatic neoplasms in male mice following currently (emphasis added) accepted nomenclature and diagnostic criteria (Maronpot, et al 1987) and to discuss the relevance, for purposes of risk assessment, of hepatic neoplasms which occurred in the study."

As a result of the PWG assessment, the tumor incidence in the Control group (Group 1) rose from

1 adenoma identified in the original study to 4 adenomas. For two of these four adenomas, the study pathologist and reviewing pathologist (the only pathologists that read all slides in the study) identified them as basophilic foci, while the other three pathologists identified them as adenomas. So, the consensus was 3 to 2 in favor of the these two adenoma designations, assuming the study and review pathologists were not convinced to vote differently at the meeting. One must pose the question, in the interest of the public health, should this level of certitude be considered acceptable where the assessment is so critical to the statistical significance of findings in dose groups? Similarly, a 3 to 2 split vote occurred in Group 3 for a number of adenoma vs basophilic foci interpretations. It should be emphasized that no carcinomas were identified in Group 1. All 4 of the lesions in question in Group 1 were identified *macro*scopically as "nodules", having dimensions at the upper end of the "small" size for liver adenomas as described in Dr. Ward's paper referenced in my letter to Dr. Hardisty. By contrast, in Group 2, of 13 *macro*scopic lesions, two were described as "nodules" having dimensions similar to those in Group 1, while 11 were described as "masses" of greater proportions, satisfying the "larger" and, in greater number, the "largest" tumor sizes as discussed in Dr. Ward's paper. A significant question is whether such large liver tumors identified after 90-104 weeks in that publication would be expected after 78 weeks, as in the malathion study. They were not evident in the control group of the malathion study. In Group 2, the original study report identified 10 mice with hepatocellular adenomas and/or carcinomas. One of these mice had a basophilic focus in addition to a carcinoma. The PWG confirmed the basoplilic focus while revising the carcinoma to an adenoma in that mouse. According to the study report, among the 10 mice involved, two mice had an adenoma and a carcinoma on differing liver lobes, a third had two carcinomas, one on each of two liver lobes and a fourth had a large carcinoma attached to two lobes, possibly, according to the PWG report, the result of two carcinomas that arose independently with subsequent fusion. In any event, whether one large carcinoma, or two that fused, this suggests an advanced stage for such a lesion for but an 18-month study. The net finding for Group 2 in the original study report was that of 10 mice with liver adenomas/carcinomas, where the number of such tumors was 13 (possibly 14), due to the presence of three (possibly 4) instances of multiplicity.

The PWG agreed with the study report on all but two carcinomas in Group 2, which were concluded to be adenomas instead. So while the study report had identified 10 mice harboring 6 adenomas and 7 carcinomas (possibly 8 carcinomas), the PWG concluded that 10 mice were affected, having 8 adenomas and 5 carcinomas (possibly 6 carcinomas). The differences of opinion among pathologists for this dose group were over the question of whether the identified lesions are adenomas as opposed to carcinomas. One basophilic focus did not enter the picture for Group 2 as explained above. As contrasted with Group 1, where two of the four adenoma calls were on a consesus 3 to 2 split vote, in Group 2, involving 10 mice (13 and possibly 14 tumors), the pathologists agreed 100% as to diagnosis of 12 tumors and split 4 to 1 on two diagnoses. Hence, the consesus was much enhanced for this group over that of Group 1.

The following views I would offer in support of my concern that a compound-related tumorigenic response may be evident in Group 2. Dr. Ward's paper, provided at the PWG meeting, appears to indicate that in the case of the B6C3F1 mouse, there may be a morphologic progression for spontaneous liver tumors from basophilic focus > adenoma > carcinoma over a 24-month (90-104 week) period, and that this progression is manifested in terms of increasing tumor size and concomitant increased likelihood and size of regions of carcinoma within the tumor. In his

publication, Dr. Ward groups the size of lesions as "small" (1-5 mm diameter), "larger" (5-10 mm diameter) and "largest" (> 10 mm diameter). In the malathion study, the Group 2 tumorigenic incidence is elevated relative to Group 1. Group 2 tumor expression appears more advanced, with several adenomas and carcinomas being identified, i.e. there appears to be a frame shift, qualitatively, in the tumorigenic expression between the two groups, Group 1 being in the basophilic > adenoma stage, with Group 2 being in the adenoma > carcinoma stage. This is also supported by the small size of the 4 adenomas in Group 1. The evidence of a more advanced stage, and hence decreased latency, in Group 2 rests with larger tumor size (where 11 of 13 lesions are described as "masses" as opposed to none being so described in Group 1, multiplicity and the absence of carcinomas in Group 1. [See discussion on latency *in* Interagency Regulatory Liason Group (1979) "Scintific bases for identification of potential carcinogens and estimation of risks" JNCI, 63, 241-268, 1979]

Interpretatively, it should be noted that any effort to separate adenomas from carcinomas, as if these were not part of a continnum in the tumorigenic response, and to treat these as independent and fundamentally different phenomena flies in the face of both the concepts expressed in Dr. Ward's paper and reason. Such a divide-and-conquer approach which diminishes the impact of the findings of both adenomas and carcinomas, should not be considered acceptable. This is probably why HED's Cancer Peer Review Committee combines adenomas and carcinomas for statistical purposes. But it really goes beyond statistics, for in the strict numerical sense, statistics for combined incidences do not quantitate the added evidence of a tumorigenic response (i.e. advanced stage, decreased latency) inherent in an increased proportion of carcinomas to adenomas, tumor size and multiplicity.

The PWG appears to conclude that a treatment-related increase of adenomas (exclusive of carcinomas) occurred in Groups 4 and 5, but not in groups 2 and 3, predicated in part on the notion that other liver histopathology (hypertropy) occurred in Groups 4 and 5 only, and in a dose-related manner. Also, according to the PWG, the adenomas in Groups 4 and 5 differ qualitatively from those in Groups 1-3, those in the latter group being held to be spontaneous in character. To the extent that carcinomas are not considered, this may be true. However, the philosophy employed here assumes *a priori* that but one carcinogenic mechanism operates in this study, which came into play only at the Group 4 and 5 dose levels. This does not consider the possibility that another mechanism operates at the lower doses. It could be <u>posed</u>, for example, that at 100 ppm, the *in vivo* concentration of malathion is not great enough to appreciably induce hepatic metabolic enzymes, or at least not to an extent necessary to meaningfully metabolize the malathion molecule, while at appreciably higher doses (800, 8000 and 16000 ppm) such induction progressively increases to the point where some protective metabolic effect seen somewhere between 100 and 800 ppm becomes progressively overwhelmed at 8000 and 16000 ppm. The liver may be so turned on and malathion so modified metabolically that a different profile of malathion derived chemical entities operate to induce tumors differently at these doses. <u>Indeed, the dose range is so enormous in this study, 100 ppm to 16000 ppm, that one would expect a substantially different xenobiotic profile to be present to affect the liver differently at the various dose levels. One cannot assume one mechanism at all doses in a study such as this on a compound with so many functional sites vulnerable to metabolic modification.</u>

Of added concern in the interpretation of this study is the questionable adequacy of the performing lab's (IRDC) historical control data base. Carcinogenicity studies performed by NTP using the

B6C3F1 mouse routinely are 24-month studies, and there is no NTP historical control data base for 18-month studies in the mouse. NTP scientists advise that studies in the mouse should be 24-month studies for adequate assessment of carcinogenicity potential. According to Maronpot et al (1987) (copy included in the PWG report), spontaneous hepatocellular tumors (particularly carcinomas) take a steep rise between 18 and 24 months in the male B6C3F1 mouse (p. 15). A low incidence of carcinomas in controls after only 18 months is not surprising, which adds to the concern for the 100 ppm malathion group, where several carcinomas were seen. The historical control data base for the performing laboratory incorporates but five control groups, of about 50 mice each, in certain instances less than 50 animals were examined. There are but four hepatocellular carcinomas in that data base. Given that several carcinomas identified in the original reading of the malathion study were interpreted as adenomas (none in reverse) by the PWG, the relevance of the four historical carcinomas for purposes of interpretaion must be questioned until confirmed by the same PWG, using the same "current" criteria.

In conclusion, evidence thus far obtained indicates that the tumorigenic response in Group 2 is a compound related effect by the OSTP (1985) definition of a carcinogen, based on increased incidence and decreased latency. Decreased latency is supported by largeness of tumors, multiplicity and high proportion of carcinomas. One mechanism of carcinogenicity across all doses may not be supportable. Additional information needed includes: 1) incidence among control mice of large (> 5mm diameter) tumors in 18- and 24-month studies; 2) microscopic examination of additional sections from the large tumors in Group 2 in order to conform absence of carcinoma; 3) PWG confirmation of carcinoma diagnoses of historical control carcinomas of the performing lab. In consideration of the findings in this study and given that NTP recommends 24 month mouse studies, was malathion adequately tested in this 18 month study?

This whole issue is of more than academic interest, for it is both surprising and of considerable concern where protection of the public health is concerned should malathion be a carcinogen at doses as low as 100 ppm. By this I mean the stakes are too high for people to say, we don't believe this could be so, in spite of the evidence, and then walk away from it.

Brian Dementi, Ph.D.
Toxicologist

cc Jess Rowland

ATTACHMENT 6

MEMORANDUM February 11, 1999

SUBJECT: Supplemental Information for the Cancer Assessment Review Committee Meeting Scheduled for February 24, 1999 to Resume Evaluation of the Malathion Carcinogenicity Data Base.

FROM: Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch
Health Effects Division

TO: Sanju Diwan, Ph.D.
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division

THRU: Alberto Protzel, Ph.D.
Branch Senior Scientist
Toxicology Branch
Health Effects Division

The Cancer Assessment Review Committee (CARC) met September 24 and October 8 and 15, 1997 to consider the malathion carcinogenicity data base. The purpose of this memorandum is to comment on the status of the work which has been pursued since the 1997 CARC meeting, and to convey all relevant documents to the CARC that have been generated since that meeting. The complete background package of DERs and other information in support of the September/October 1997 meeting remain in the hands of the committee and will not be re-submitted under this memorandum.

No complete report of the results of the 1997 CARC meeting deliberations and conclusions was ever produced by the committee. However, certain additional testing requirements were imposed as set forth in the November 3, 1997 memorandum of Jess Rowland, CARC Executive Secretary, a copy of which is appended. (Attachment 1) In summary, the CARC requirements included: 1) a Pathology Working Group (PWG) assessment of the male mouse liver tumor response in the recently submitted mouse carcinogenicity study (MRID 43407201); 2) full pathology assessment of nasal tissues from the same mouse carcinogenicity study, a tissue site not examined in the original pathology evaluation; and 3) pathology re-evaluation of nasal, pituitary and uterine tissues in the

recently submitted malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901).

As to the status of fulfillment of these particular requirements, the PWG assessment of male mouse liver tumors, performed by Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, has been completed. The PWG's May 8, 1998 report (MRID 44554901) and HED's January 27, 1999 review of the same are here forwarded to the CARC. (Attachments 2 and 3, respectively)

The pathology report (MRID 44733501) of evaluations of nasal tissues from the mouse carcinogenicity study dated January 8, 1999, received in HED February 1, has not been reviewed. HED awaits the registrant's submission of a missing summary table of the findings, expected soon, before drafting a final review of the submission. The pathology assessments of the pituitary and uterus from the rat study, received in HED January 27, have been submitted to HED's pathologist for comment, following which a brief statement of the findings for both tissues will be rendered within HED. This is not expected to be a time consuming matter. The pathology re-evaluations of the nasal tissues from the rat study have not been received by the Agency as of this date. The reviewing pathologist has advised HED the report is imminent. (Attachments 4, 5 and 6 reserved, respectively, for these outstanding submissions)

Following the Agency's receipt of the male mouse liver PWG report, the CARC re-convened June 10, 1998 to consider those particular findings. This meeting was an expedite that occurred prior to full HED review of the PWG report. While no official report of this meeting of the CARC was produced, as best remembered the committee's conclusions were that the PWG report should result in no immediate change in the regulatory status of malathion, and that final decision on interpretation of the study be deferred until such time as all other outstanding work has been received and final review of the malathion carcinogenicity data base is undertaken by the CARC.

Not long after the September/October, 1997 CARC, the HED reviewer/presentor of the data base to the CARC, Dr. Dementi, submitted a memorandum dated November 26, 1997 to the CARC Chairman, taking issue with certain decisions rendered at the meeting. A copy of that memorandum is here being conveyed to the CARC for consideration. (Attachment 7) Furthermore, in connection with his concern over the lack of a full report of the results of the 1997 CARC meeting, i.e. complete minutes of the meeting, Dr. Dementi expressed his views in a January 19, 1998 memorandum to Mr. Steve Johnson, then Acting Director of OPP. (Attachment 8)

Also, since the September/October 1997 CARC meeting, leukemia and interstitial cell testicular tumor incidence data from the malaoxon combined chronic toxicity/carcinogenicity study (MRID 43975201) have received statistical re-evaluations by the registrant at HED's request, the results of which are here being communicated to the CARC. (Attachment 9)

An issue previously before the CARC was that of the response of nasal tissues in the combined chronic toxicity/carcinogenicity studies in the rat. It may be of value for the CARC to have in hand the results of the subchronic inhalation study on malathion, which received particular attention by the HIARC in its December 22, 1998 report. (Attachment 10, selected pages from the 12/22/98 HIARC report) The HIARC is requiring another subchronic inhalation study, most particularly to identify

the NOEL for nasal tissue hyperplasia/degeneration and cholinesterase inhibition. The HIARC had been made aware at its last meeting of a 2-week range-finding inhalation study in the rat, not previously submitted to the Agency, performed by the registrant for purposes of dose selection in the subchronic inhalation study. The range-finding study demonstrated nasal hyperplasia/degeneration and cholinesterase inhibition, at all doses, after only two weeks of treatment. So when the CARC considers nasal tissue effects in the chronic oral studies, findings in the inhalation studies may be instructive in the interpretation. The relevant subchronic inhalation DER was present in the background package provided for the September/October 1997 meeting of the CARC. The question of carcinogenicity as it may relate to the microscopic lesions of nasal passages was raised in the DER (p. 2). Nasal tissue findings in the 2-week range-finding inhalation study are summarized in a March 10, 1998 memorandum of Brian Dementi to Jess Rowland, HIARC Secretary. (Attachment 11)

ATTACHMENT 7

Leukemia - Recommendation To CARC For Further Assessment/ B. Dementi-2/24/99

Rationale ***(not defining, but offered for CARC consideration):***

1978 NCI Study in F344 Rat (male/leukemia):

Dose (ppm):	0	2000	4000
Leukemia Incidence:	13/50 (26%)	20/50 (40%)	8/49 (16%)
Survival (103 weeks)	54%	28%	0%

The PWG report of an assessment performed at NTP (Huff et al, 1985), which considered leukemia along with other tumor end points, says: 1) reduced survival made interpretation of leukemia incidence difficult; 2) life-table analyses suggest increases in leukemia, primarily in the low dose group (statistically significant); 3) life-table analyses discounted by NTP because leukemia was not a cause of death (death attributed to "chemical toxicity"), and 4) increase was not significant by incidental tumor tests or Fishers Exact Test, and, hence, not related to treatment.

Comments: 1) mortality does pose a problem for interpretation, since where animals die early they are at less risk of developing the condition, and also competing toxicity may preclude expression. This is evident in the recent 1996 F344 rat study, wherein survival among males at 24 months in the control, 100/50, 500, 6000 and 12000 ppm groups equalled, respectively, 67%, 75%, 53%, 26% and 0%. Number of male rats among 55 rats per group with leukemia (death due to leukemia) across the same dose groups were, respectively,: 23(7), 16(7), 24(14), 18(13) and 1(1). Along with leukemia, chronic nephropathy was a principal cause of death. In the same respective order, the number of rats with chronic nephropathy (death due to nephropathy) were: 54(2), 54(2), 54(4), 55(23) and 55(47). These numbers indicate that in group 3, deaths due to leukemia (14) exceeded deaths due to nephropathy (4), while in group 4, deaths due to nephropathy (23) exceeded deaths due to leukemia (13), suggesting that competing toxicity has impeded expression of leukemia in group 4, a phenomenon which becomes more full blown in group 5, where nephropathy claimed 47 rats, while but one leukemia was observed, which was identified as the cause of death. It is perhaps noteworthy that in this case, among rats harboring leukemia, the percent that succumb to the condition increased with dose, 7/23 (30%), 7/16 (44%), 14/24 (58%), 13/18 (72%) and 1/1 (100%), respectively, for control and dose groups in increasing order. **Points being made here are 1) that leukemia was a cause of death among male F344 rats in the new study, which suggests it may have been a cause of death in the 1978 NCI study, and cause of death should be evaluated more closely in that earlier study if critical to choice of method of statistical analysis as claimed in Huff et al; 2) competing toxicity and resulting increased mortality does (did) compromise the tumorigenic response, so indeed as noted in Huff et al and as would be true in the recent study, reduced survival makes interpretation difficult in both the 1978 and 1996 studies. Is leukemia properly evaluated in these studies? Would a dose somewhere between 500 and 6000 ppm have been more useful for assessing leukemia response?**

Other reasons to evaluate leukemia response more closely: 1) Increased leukemia incidences among females in the 1996 F344 rat study, where high mortality in the highest dose group may have compromised expression; 2) HED's Peto test was positive for females at 100/50 ppm ($p = 0.025$) and

close at 500 ppm (p = 0.059); 3) MRID study submission statistician's report positive by Fishers Exact Test and Cox or G-B Test at 100/50 and 500 ppm (p. 638) but says, "Finding not supported by dose-response." Among male animals in the 1996 study, the study statistician's report found a positive trend for leukemia in males ($p \leq 0.01$) (p. 638), and claims that "....number of tumors (leukemia) in the higher doses were greater than expected, and were thus judged to have statistically more tumors" (p. 636). 3) Increased incidence of lymphoma in males in NCI's (1979) malaoxon study in F344 rats (incidences were: 0/50, 0/50, 4/50 @ 0, 500, 1000 ppm) Tox Branch calculation revealed positive trend (p = 0.006) and high dose pairwise (p = 0.059). The Exact Test for Trend also positive (p = 0.0114). In some cases, lymphoma and leukemia are combined by NTP; 4) Increased incidence in male F344 rats in the 1996 malaoxon study, as presented in Attachment 9 in CARC package.

ATTACHMENT 8

To: Jess Rowland, Executive Secretary, Cancer Assessment Review Committee April 1, 1999
From: Brian Dementi, Ph.D, Toxicology Branch I

In reference to your March 24, 1999 draft report of the various CARC meetings on malathion, I find that constraints of time in concert with the complexity of the subject precluded my developing a response by March 31 that I would consider satisfactory. However, since you say this is but a summary draft and that a comprehensive report will come later, at which time you say discussions and deliberations of the CARC will be provided, my more detailed response will need wait until that time.

In rendering comments, I will refer to your draft by page number and paragraph.

1) p. 1, paragraph 1: You might also acknowledge that the results of the National Cancer Institute studies performed in 1978-79 were before the committee, in abbreviated or summary form. These studies must be recognized for what they may contribute to the overall assessment.

2) p. 2, paragraphs 1 and 2: Richard Brown is with the Agency's Institute for Individual and Organizational Excellence.

3) p. 2, paragraphs, all: I would note the considerable flux in participants. Persons who were present at all meetings were: Burnam, Rowland, Ioannou, Copley, Stewart, Taylor and Dementi.

4) p. 4, paragraph 7: The study pathologist is listed in the original MRID study submission as Dr. William Wooding.

5) p. 5, "Carcinogenicity study in B6C3F1 Mice: Prior to offering specific comments on this topic, I fell it imperative to raise a question. Upon re-examining the 1996 EPA Proposed Cancer Assessment Guidelines, I do not find a definition of a carcinogen. Nonetheless, these Guidelines do frequently cite the Office of Science and Technology Policy (1985)(OSTP) publication, which is entitled: "Chemical Carcinogens; A Review of the Science and its Associated Principles, February 1985", and thus presumably considers it to be authoritative. This OSTP publication says "**A chemical carcinogen may be a substance which *either* (emphasis added) significantly increases the incidence of cancer in animals or humans or significantly decreses the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen**." (p. 10415). I am curious to know whether the CARC affirms this concept of a carcinogen?

6) p. 5, paragraph 4: Typo, hypertrophy seen at **8000** and 16000 ppm

7) p. 5, last paragraph: I have difficulty accepting the notion that cholinesterase inhibition in the absence of clinical signs should be used to conclude a dose level is excessive for carcinogenesis evaluation, and recommend this issue to SAP, or another External Peer Review. My views are expressed in a November 26, 1997 memorandum to William Burnam, CARC chairman, following the 1997 CARC meetings. In all humility, I submit that my letter should have been acknowledged and responded to by the CARC. Time remains to do so.

8) p. 5, paragraph 3, carcinogenicity study in B6C3F1 mice: Since your report does get into an interpretive treatment of the mouse liver tumor response, I must offer the following alternative assessment that may help the committee with its conclusions, or alternatively serve as a basis for a minority report.

The draft report is too restrictive to statistical considerations of the data, and not sufficiently reflective of the biological character (evidence of decreased time of tumor development or decreased latency, a defining characteristic of a carcinogen according to the OSTP (1985) definition) of the response, particularly in the low dose male group, that was presented to the committee in an extensive written review of the PWG report. (Attachment 3 to the February 24, 1999 CARC meeting package) The latter, though presented at the meeting and should be acknowledged in these minutes, was consistent with a full and adequate assessment of the tumorigenic response according to various cancer guidelines. The concepts that were presented at the meeting that should be reflected in these minutes are summarized as follows:

a) this study was conducted as required by the 1990 HED Cancer Peer Review to address an equivocal combined hepatocellular tumorigenic response among male mice at the high dose level ($p = 0.031$, pairwise) (pooled control: 8/49, low dose: 7/48, high dose: 17/49) in the 1978 NCI study (doses tested : 0, 8000 and 16000 ppm). I might add, there was no increase among females (pooled control: 3/48, low dose: 0/49, high dose: 2/47). The Agency required a new study, testing at the same dose levels to resolve the equivocal finding at 16000 ppm among males. The new study revealed not only a very high tumorigenic response at 16000 ppm among males (96%), but among females as well (84%) versus 2% in control groups of both males and females as presented in the original study report. There is no explanation for the substantially higher incidence among males, and the remarkable response seen among females for the first time in the new study. Also, as contrasted with the NCI study, the tumorigenic response was elevated in both sexes at 8000 ppm. Again there has been no explanation for these contrasting findings. Both studies, nonetheless, reveal the liver as a target organ;

b) multiplicity was of higher incidence (4 mice) in the 100 ppm male group than is shown in the CARC draft table (2 mice) (your p. 11), characterized as follows: livers with one adenoma plus one carcinoma (2 mice) and livers with two carcinomas (2 mice, one being equivocal);

c) in terms of the natural history of neoplasia (foci of cellular alteration > adenoma > carcinoma) [e.g. Maronpot et al (1987)], the response in the low dose group is more advanced (adenoma > carcinoma) than in control (basophilic foci > adenoma), wherein successively the original study pathologist saw 1 adenoma and 3 basophilic foci, the PWG's reviewing pathologist saw 2 adenomas and 2 basophilic foci and finally the full PWG interpreted all 3 basophilic foci as adenomas, yielding 4 adenomas; all interpretations considered suggesting the earliest stage in the "natural history of neoplasia", i.e. small lesions, most just emerging the basophilic focus stage into the adenoma stage, no carcinomas), suggesting decreased latency or a more advanced stage of tumor development in the low dose group;

d) the dose range in the study, 100 ppm to 16000 ppm is so wide, that one cannot presume, or impose upon the interpretation of this study, a common mechanism of carcinogenicity operating

across all doses, thus the unexpected *absence* of carcinomas among so numerous adenomas at 16000 ppm, suggesting a different mechanism at this dose, cannot be used to discount carcinomas at the lowest dose on the grounds of their not being dose related. A different phenomenon evidently is occurring at the higher doses that does not involve progression of adenomas to carcinomas, at least after 18 months. One must entertain the prospect of more than one mechanism of carcinogenicity across such a wide dose range;

e) the weakness of the historical control data base, containing but five studies, and the 4 mice with carcinoma in those control groups did not receive the same scrutiny for revised diagnosis that carcinomas in the malathion study met at the hands of the PWG, which resulted in the reduction of the total number of male mice with carcinomas in the study from 16 to 8, presumably using new and improved criteria for diagnosis that, again, were not applied to the total of 4 carcinomas in the entire historical data base;

f) inadequate sectioning of large adenomas in the low dose group to confirm absence of carcinoma regions (trabecular formations) once 6 carcinomas among the four mice with carcinoma in that group were identified, i.e. logically the need for multiple sectioning is not indicated for a group wherein no carcinomas were identified by single sectioning of small tumors, but the converse should be held to be true for a group wherein tumors are large, carcinomas have been identified in a goodly number by single sectioning, and questions of statistics, and latency hinge on definitive assessments of the nature of the tumorigenic response. (Note: McConnell et al (1986) says as one reason to combine malignant and benign tumors is because "...time and resources do not allow for step-sectioning (multiple sections) of a given "benign" lesion to determine whether malignant areas are present." p. 284) Yet, "time and resources" in this case should not be a consideration for assessment of a particular end point so critical to the protection of the public health. Hence, if the findings at 100 ppm in this study are not to be considered as positive evidence of carcinogenicity with the data already in hand, there must be further sectioning of all adenomas in the control and low dose groups to rule out the possibility of yet additional evidence of a more advanced tumorigrenic response in the 100 ppm group versus control. This should be recognized as a critical element of this analysis given the closeness of statistical significance and the need to know more as to the magnitude and stage of development of the tumorigenic response in the 100 ppm group;

g) largeness of tumors in the low dose group versus control as identified macroscopically;

h) some people tend to discount findings in this study at 100 ppm claiming an increase was not seen at 800 ppm. Yet, there was a numerical increase in that group, though not statistically significant, but a change of mechanism somewhere along the road between 100 and 8000 ppm may explain a valley wherein metabolic processes are more adequate to mitigate tumorigenic responses occurring by different mechanisms at lower and higher doses; it is noteworthy that EPA's 1996 Guidelines say: "A strong response relationship across several categories of exposure, latency, and duration is supportive for causality given that confounding is unlikely to be correlated with exposure. The absence of a dose response relationship, however, is not itself evidence against a causal relationship." (p. 47);

i) various guidelines, including EPA's 1996 Guidelines, instruct that one must consider the

linearity of the response. EPA's Guidelines say: "A default assumption of nonlinearity is appropriate when there is no evidence of linearity and sufficient evidence to support an assumption of nonlinearity and a nonlinear procedure."(p. 67) These guidelines also suggest a margin of exposure assessment be used in the case of nonlinear responses.

j) See quotation from Agency's 1996 draft cancer guidelines (p. 27) as rendered in my November 26, 1997 memorandum to the CARC chairman.

All of this information, if not summarized in the CARC report as having been considered, becomes lost to consideration by readers who merely read the report and do not pursue the background documents. The committee needs to *explain away* these concerns brought before the it in order to substantiate its conclusions. Furthermore, the committee has not explained how 800 ppm in this study can serve as an adequate dose, being so far below the limit dose, if 8000 ppm, which is close to the limit dose is discounted. In other words, absent the 8000 and 16000 ppm dose groups, the study is inadequate to assess carcinogenicity at sufficiently high doses to satisfy the Guidelines and sound principles for assessment of a chemical's carcinogenicity.

9) p. 5, paragraph last: You should cite any known document that instructs at what level of cholinesterase inhibition (and which enzyme) a dose group should be discounted on the grounds of excessive toxicity, and if this has not been endorsed by the SAP or other external peer review group, I recommend it be done. I do not accept that brain cholinesterase inhibition at the levels shown in this study are sufficient, in the absence of clinical signs, to preclude testing a compound's carcinogenicity. I should note, though, that the EPA Guidelines do say that clinical chemistry effects can be used to identify excessive dosing. (p. 50) However, little further qualification is offered, and the statement does not speak specifically to cholinesterase inhibition. Testing up to and including a limit dose should be conducted if well tolerated in order to evaluate a compound's carcinogenicity potential, given the small number of animals under study, their shorter than human life time, and all other reasons that historically have been invoked to secure optimum sensitivity for carcinogenicity potential. Since the study was conducted at 8000 and 16000 ppm to emulate the earlier NCI study, and to address a question raised by that study, the data should not be discounted because the doses slightly exceed a limit dose, which in itself is arbitrarily established and is now much lower than 5% of the diet previously considered a maximum dose as still described in HED's carcinogenicity testing procedures.

10) p. 9, paragraph last: You say, "The CARC also discussed the "multiplicity" of the component of liver tumors in tumor bearing animals (i.e., the presence of adenomas and carcinomas in the different lobes of the liver in the same mice)." Yet, you do not indicate the degree of multiplicity in your text and the table on p. 11 does not convey the degree of multiplicity for the low dose group that was evident. There were two mice in the group with an adenoma and a carcinoma on differing lobes, a third with carcinomas on two lobes and a fourth with a large carcinoma spanning two lobes which may have been two that subsequently fused.

11) p. 11, paragraph 3: You say, "Dr. Brennenke, the consulting pathologist commented that in the evaluation of carcinogenicity, 'tumor bearing animal' counts as one regardless of the number of multiplicity of any tumor type." I would agree that is true when limited to a class of tumors such as

hepatocellular adenomas and carcinomas. Your quote continues, "Therefore, a tumor bearing animal with 'multiple' tumors should not be given any more weight than a tumor-bearing animal with a 'single' tumor." I am not certain what Dr. Brennenke actually said, but I suspect he meant by this latter quote that single versus multiple tumors do not alter the statistical treatment of the data in terms of number of animals affected. However, your quote goes to the heart of the matter as to the distinction the OSTP (1985) makes in its definition of a carcinogen, namely, incidence and latency, or tumor progression in defining a carcinogen. Multiplicity most certainly weighs into the assessment of the latency or the state of progression aspect of carcinogenicity. There are many sources I could cite in support of this, and this has been documented in HED's review of the PWG report presented to this committee. McConnell et al (1986) say in a concluding remark on the principles of carcinogenesis assessment, "The greatest weight of evidence consists of a dose-related induction of multiple malignant neoplasms with a shortened latent period." (p. 288) This same reference also says: "A particular chemical that induces a statistically significant shift in tumor expression from benign to malignant *without the total incidence increasing* (emphasis added) may be regarded as a carcinogen." (p. 283). How does one evaluate that statistically when there is evidence of both such a shift and an increase of incidence. As another example, OSTP (1985) says: "In addition to statistical significance (Sec. D), there should be biological significance as well. The use of a variety of biological information on dose response, tumor progression, tumor latency, *tumor multiplicity* (emphasis added), findings in other studies, etc. can add confidence to the final assessment." (p.10415). So an animal exhibiting 'multiple' tumors is indeed to be given more weight than a tumor-bearing animal with a 'single' tumor. I think you need to clear you attribution to Dr. Brennenke with him.

12) p. 13, paragraph 2: You might indicate that mortality in males at 6000 ppm was also quite high, 74%. Also, the last sentence should show NOAEL = 50 ppm rather than 500 ppm.

As discussed at the HIARC meetings, in females a NOEL (NOAEL) for erythrocyte cholinesterase inhibition was not identified at the 3-month time point where dosing had been at the dietary level of 100 ppm. A NOAEL was subsequently identified with the lowering of dose from 100 to 50 ppm. Given the potential for adaptation and the fact that erythrocyte cholinesterase inhibition in females at 500 ppm seen at 3 months also returned to the control level at the 6 months time point, it cannot be claimed that inhibition in females would not have been seen during an initial 3 months dosing at 50 ppm, a critical time frame.

13) p. 13, paragraph 3: Suggested sentence change: "At the September 24, 1997 meeting, the CARC concluded that the 12000 ppm dose in both sexes (**due to mortality**) ....."

Also you say the CARC agreed that the 500 ppm in males was adequate to assess the carcinogenic potential of malathion. This is another issue requiring SAP or other external peer review assessment. As revealed in this study, the F344 male rat appears to be particularly vulnerable to increased mortality due to chronic nephropathy in response to malathion, which resulted in excessive mortality at the top two doses, and thus precluded adequate testing for carcinogenicity in males at 6000 ppm. This means the proper assessment of carcinogenicity in male rats at or near an MTD was not, and indeed likely cannot be, achieved in this strain of rat. Hence, the study should be considered unacceptable insofar as it is considered negative for carcinogenicity in male rats. The same argument is not applicable to females.

14) p. 14, paragraph 2: While it is true there were no statistcally significant increases in hepatocellular tumors at any dose level, as explained above, the study is inadequate to evaluate liver tumorigenic response in males. Furthermore, a tumorigenic expression at the higher doses may have been obviated by early death, i.e. sufficient animals were not at risk. Also, the well recognized principle of competing toxicity may have precluded a tumorigenic response in males at the top two doses, and for that matter in females at 12000 ppm. This study cannot be accepted as a negative for hepatocellular tumorigenesis.

15) p. 14, paragraph 3: Typos: the incidence of adenomas at 12000 ppm is **10%** rather than 7%; the incidence of carcinomas is **8%** rather than 16%. Just as you provided the mean (1.6%) historical control incidence here, you should do likewise for the male mouse liver carcinoma historical incidence. It is notworthy that in F344 female rats hepatocellular carcinoma is considered a rare (< 0.1%) tumor type in the NTP data base.

16) p. 15, paragraph 1: SAP or other external peer review should address acceptability of hepatocellular tumorigenic response at 12000 ppm in females.

17) p. 16, paragraph 8: The term is **respiratory epithelium** as opposed to "respiratory tract". You do not mention the even greater rarity of neoplasms of the olfactory epithelium as discussed at the February meeting. Also, there is no mention of the rare oral cavity squamous cell tumors, identified in the same set of nasal tissue slides, noted both in the original study report (2 rats) and in Dr. Swenberg's letter (3 rats).

18) p. 17, paragraph 4: As stated previously, excessive mortality in the high dose male groups renders the study unacceptable for the evaluation of this tumorigenic end point. Even so, the increased incidence actually seen at 6000 ppm cannot be discounted, and further it may underestimate the expression of this tumor as the result of competing toxicity and reduced animals at risk, and reduced time at risk, due to the survival problem. The issue of the acceptability of the male component of this study awaits SAP or other external peer review.

19) p. 18, paragraph 4: Should the first sentence read "Based on the overall data for this tumor type, the CARC concluded February 24 that ....."?
As I have indicated earlier, if a CARC report covering the meetings in the Fall of 1997 had been written, we could quite possibly have had more of this behind us. Compromising my response is that lapse of time during which memory tends to fade concerning many important ideas that were before us at the time. Your mentioning the NCI studies here is acknowledged.

20) p. 19, paragraph 4: Suggested language: **It was noted at the CARC meeting that in the 6000 ppm group females, carcinoma incidence on re-evaluation declined to 1 from the 4 diagnosed originally, even though more animals were examined in the re-evaluation. This decline evidently is attributable to more restrictive criteria for carcinoma diagnosis employed in the re-evaluation.** Based on histopathological examination of all animals, the CARC concluded that the pituitary tumors are not attributable to treatment.

21) p. 21, table first: While it is certainly implicit this data is from the original study report, should

it be labelled as such?

22) p. 21, table second: Corrections: deciduoma, 1 incident in group 2; endometrial carcinoma, 1 incident rather than 2 incidences in group 3.

23) p. 22, Testicular tumors: In my November 26, 1997 memorandum to the committee chairman following the CARC meetings in September/October 1997 as mentioned here previously, I also raised a question concerning the CARC's assessment of this tumor type. As stated previously, I would have hoped the committee would acknowledge that letter and attend to the issues raised, but that has not been the case. With respect to testicular tumors, I expressed the view that the committee re-visit this topic, as I am concerned the findings were dismissed too quickly by the committee. Yet, when a committee member mentioned this subject near the end of the February 24, 1999 meeting, any further pursuit of the matter was quickly dismissed with the assertion that the matter was settled at the earlier meetings. This denial of my legitimate written request, in addition to the oral request by a committee member in February, strikes as rather authoritarian. Again, in all humility it is my request that the committee discuss my letter of November 26, and respond to it as being a legitimate document before the committee.

My brief comments where this tumor type is concerned are summarized as follows:

a) The MRID study report's statistician concluded: "The material is associated with increased interstitial cell testicular tumors in male rats at *all doses* (emphasis added) measured based on Haseman's and Fisher's test; Haseman's at the 12000 ppm dose group, Fisher's at the 50, 500 and 6000 ppm dose groups." (p. 5346) Furthermore, earlier in this statistician's report he says this tumor type ".....indicated a statistically significant dose related increase in tumors corrected for suvivorship at least at the 5% level of significance."; and ".....the number of tumors in the higher doses were greater than expected, and were thus judged to have statistically more tumors." (p. 5345). He explained the tests (Cox and Gehan-Breslow) used in the latter case assume the tumor was the cause of death (fatal tumor context), and therefore resorted to the Haseman and Fishers tests as mentioned above. The point here is the study statistician having employed his expertise, has concluded there is an increased incidence of testicular tumors at all doses. **My question to ths CARC would be, has any other authority in the area of statistical analysis refuted the study statistician's statistical approach or conclusions. If not, then please advise on what authority the committee discounted his conclusions.** This requires clarification.

b) The Draft says on p. 22: 1) <u>the statistical increase in tumor incidence at the top and penultimate dose levels *could* (emphasis added) be due to high mortality at these doses</u>. This is speculation, hardly the sort of rationale required to discount the findings; 2) <u>sufficient data are not available to determine if there was decreased latency.</u> Such data obtained from serial sacrifice is rarely available in the Guideline studies. There is a clear implication in the statistician's report of decreased latency as the explanation for the statistically significant increases in the face of nearly the same incidence across all doses. Therefore, you place the burden of proof in the wrong corner. Conservatively and in the interest of the public health, the data should be treated as illustrating decreased latency until proved otherwise. I remind the committee of the OSTP (1985) definition of a carcinogen which includes that of an agent which ".....significantly decreases the time it takes a naturally occurring

(spontaneous) tumor to develop relative to an appropriate background or control group." (p. 10414); 3) this tumor type is not useful in overall evaluation since its occurrence are similar at all dose levels. If the tumors occurred sooner than those occurring spontaneously, it fits squarely into the second component of OSTP (1985)'s definition of carcinogen.

c) At the CARC meeting in September/October 1997, I understood a committee member to say these tumors are of questionable concern because they are benign, and rarely seen as malignant. I took it for face value, did not record it and don't remember who spoke it. However, I now find these tumors may be of somewhat greater concern. Quoting from McConnell et al (1986): "For some neoplasms there is substantial evidence for the sequential progression from hyperplastic to the benign stage and from the benign to the malignant stage. Progression has been suggested for the following lesions: epidermal skin lesions in mice, alveolar lesions in mice, esophageal and forestomach lesions in rats, bladder urothelial lesions in rats, *testicular interstitial cell lesions* in rats, and prostatic lesions in rats." (p. 284) So concern does reside with these tumors that they may advance, and I would suspect more likely so in the animals life time the sooner they appear I should note this quote continues: A direct transition from hyperplastic or dysplastic to the malignant stage has been suggested for *nasal cavity,* glandular stomach and *thyroid C-cell lesions* in rats, although others do recognize adenoma as an intermediate stage in the development of thyroid C-cell carcinomas." (p. 284)

So, in summary the committee needs to discuss this further, and perhaps it also is a matter for external peer review.

24) p. 22, Leukemia: This subject we anticipate will be discussed further by the CARC

25) p. 24, paragraph 4: At the Feburary 24 meeting, the committee was presented with a statistical reanalysis of the leukemia incidence data in the malaoxon study. This statistical analysis was performed by the registrant at HED's request, the review of which was dated May 19, 1998 and was attachment 9 in the CARC package. As analyzed by Peto's test, for mononuclear cell leukemia in males there was a positive trend ($p = 0.03$) and a positive pairwise comparison ($p = 0.05$) for the high dose group (2000 ppm) versus the control group. There was no significant increase among females. Hence, the draft report should acknowledge this finding rather than say: "There was no evidence of carcinogenicity in male or female rats."

26) p. 24, paragraph 6: suggested revision to read: Independent review and analysis of the re-read data submitted to the Agency, but which initially was available to the committee only in a letter format from Dr. James Swinberg. The original study pathologist listed as Dr. William Wooding.

I have not had a chance to offer any comment on the draft report's log of voting results.

I should note that in the case of the malathion F344 rat study, dosages expressed in terms of mg/kg/day shown on your p. 13 should be revised to reflect recalculations of these values per my March 8, 1999 memorandum to you.

I am not satisfied with these comments I am submitting to you. I consider it important to respond to your request for comments, but the time frame is too short for such a complex subject. I would

want more time to proof read this, and modify its content. There is more that needs to be said. I trust there will be that opportunity later when a draft of a final report is prepared and when fewer differences of opinion remain. Therefore, I would be pleased if you would use it as an aid, but it not be circulated outside the committee, or at least until I had more opportunity to revise it. I simply cannot do more at this time.

ATTACHMENT 9

Mr. William Burnam, Chairman April 27, 1999
Cancer Assessment Review Committee
Health Effects Division

At the last Cancer Assessment Review Committee (CARC) meeting held February 24 to consider the malathion carcinogenicity data base, I petitioned the committee both orally and in the form of a memorandum (Addendum # 3) (copy appended) to engage further assessment of the leukemia data. Also, please recall that following the September/October 1997 meetings of the CARC, in my November 26, 1997 letter to you as chairman (copy appended), I made a similar though more brief petition that, among other matters, the CARC re-visit the leukemia data.

Accordingly, at the last meeting, the CARC elected to reconsider the leukemia data, and Ms Lori Brunsman has been provided the data from the February 27, 1996 combined chronic toxicity/ carcinogenicity study in the R344 rat (MRID 43942901) for purposes of statistical analysis.

In order to facilitate my appreciation and understanding of leukemia as a process, I discussed the subject with an expert pathologist at the National Toxicology Program (NTP), Dr. Robert Maronpot. Specifically, I was curious as to whether in the onset of leukemia there may be distinct stages analogous to those constituting the "natural history of neoplasia", characteristic of other neoplastic processes such as that of hepatocellular tumorigenesis, wherein there is a generally recognized sequence of events or progression: hyperplasia > adenoma (benign stage) > carcinoma (malignant stage). Dr. Maronpot affirmed the complexity of leukemia as a disease process, but said once leukemia is diagnosed, it is a recognized malignancy. The progression of leukemia, which can lead to death, is assessed by the degree of invasion of many tissues such as spleen, liver and lungs. Death attributable to leukemia is diagnosed on the basis of incapacitation and destruction of such vital organs as those of the liver and lungs. He explained further that in certain cases where leukemia is diagnostically a close call, NTP will go back and study more closely tissues from various organs to help confirm a diagnosis.

In the absence of clear stages of tumorigenesis with which to gauge progression or development of a leukemogenic response such as that of hepatocellular tumorigenesis, I posed the question as to whether increased mortality due to leukemia would constitute evidence of advancement or development of leukemia, i.e. would treatment-related increased proportions of animals dying of leukemia among those diagnosed with leukemia, serve to establish treatment-related progression of leukemia? *This NTP pathologist's response was clearly in the affirmative*. Furthermore, he explained that such evidence, even in the absence of any treatment-related increased incidence of leukemia, would in his words pose "a bad situtation for the chemical". NTP would consider such information, assuming its validity, as constituting positive evidence of carcinogenicity under the concept of increased tumor progression, or decreased latency. Of his own volition, Dr. Maronpot vounteered that NTP considers increased progression or decreased latency as a basis for classifying or labelling a compound as a carcinogen. He went on to elaborate that NTP considers both treatment-related increased incidence and/or progression as defining a carcinogen. I then mentioned the Office of Science and Technology Policy (OSTP) (1985) definition of a carcinogen, which he

affirmed as consonate with NTP's operating principle. As a matter of interest, the OSTP (1985) definition reads: *"A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen."* (p. 10414-10415) Dr. Maronpot went a step further in elaborating NTP's approach to identifying carcinogens by saying NTP would also consider as positive evidence of carcinogenicity the finding of a few very rare tumors in a particular study.

Please note that in my appended February 24, 1999 recommendation for CARC's further assessment of leukemia, you will find for the 1996 combined chronic toxicity/carcinogenicity study in the F344 rat the incidences of leukemia, along with the number of deaths attributed to leukemia, and the percentages of animals diagnosed with leukemia that died of the condition. I should remind the committee that leukemia was a principle cause of early death in this study, especially among male rats in the 500 and 6000 ppm dose groups. For example, among males at 6000 ppm, in which group mortality was 74%, leukemia accounted for essentially twice the number of deaths as in the control, despite the fact its incidence may have been compromised by enhanced mortality due to chronic nephropathy.

Where incidence is concerned, the interpretation among male rats is rendered difficult by high mortality in the 6000 ppm (74%) and 12000 ppm (100%) dose groups. It is most evident that leukemia incidence at 12000 ppm was compromised by early mortality and/or competiting toxicity such that but one animal was diagnosed with leukemia as compared with 23 in the control group. Furthermore, I would venture to say that increased mortality and/or competing toxicity likewise compromised full expression of potential treatment-related increased leukemia incidence in the 6000 ppm group and possibly, though probably less so, in the 500 ppm dose group as well. Proper statistical treatment of the data should indicate whether, in consideration of increased mortality, incidences in dose groups were higher than expected, but as I understand it, statistical analysis cannot tell us what the incidences in dosed groups would have been had survival been more normal. However, setting the incidence question aside and looking to the question of progression as providing evidence of carcinogenicity, the data show a treatment-related increase of death ascribed to leukemia among animals diagnosed with leukemia. The number of male rats among 55 rats per group diagnosed with leukemia (death due to leukemia) were 23(7), 16(7), 24(14), 18(13) and 1(1) for the control, 100/50, 500, 6000 and 12000 ppm groups, respectively. Hence, among rats diagnosed with leukemia, the percentages dying with leukemia were: 7/23 (30%), 7/16 (44%), 14/24 (58%), 13/18 (72%) and 1/1 (100%), in the same respective order.

In my view, the CARC must seriously consider whether the above data indicating a treatment-related increase in proportions of animals dying of leukemia among those harboring the condition satisfies as evidence of carcinogenicity based on increased progression (decreased latency), under the OSTP (1985) definition, irrespective of whether there is increased incidence or adequate data even to assess incidence due to leukemia. It is noteworthy that in terms of treatment-related progression of leukemia to lethality among male rats, this data does not identify a NOAEL.

I should advise that leukemia and chronic nephropathy were the principal causes of early death among

male rats in this study. I am unwilling to speculate, but CARC may wish to consider the question of whether chronic nephropathy, as a principal cause of death in this study, especially at the top doses among male F344 rats, in any way compromised the definitive assessment of the leukemogenic potential of malathion.

With regard to the 1979 NCI study in the F344 rat, as discussed (mistakenly as 1978) in the appended 2/24/99 memorandum of B. Dementi, the fact remains that Huff et al (1985), using life-table analysis concluded leukemia was of increased incidence among male F344 rats, but discounted this finding on the grounds that leukemia was not a cause of death, and, hence, life table analysis was inappropriate. Huff et al (1985) attributed death nonspecifically to "chemical toxicity". On reading Huff et al (1985) and the National Cancer Institute (1979) reports, I do not locate any stated causes of death other than the general non-specific and unexplained attribution to "chemical toxicity" in Huff et al., and certainly no assigned causes of death on an individual animal basis as was done in the 1996 study. The more recent (1996) study in the F344 rat clearly identified death due to leukemia in a treatment-related manner among rats diagnosed with leukemia. Doses in the recent study: 0, 100/50, 500, 6000 and 12000 ppm clearly interlace with doses in the 1979 study: 0, 2000 and 4000 ppm, such that if leukemia were a treatment-related increased cause of death, essentially across all doses in the recent study, it likely was so in the 1979 study. I submit that in the absence of more definitive assessments of causes of death in the latter study, beyond that of simply "chemical toxicity", where the immediate cause of death could be due to anything, it is not unreasonable to be seriously concerned that leukemia was a cause of death in the earlier study, in which case there was a positive finding by the proper method, life-table analysis, *in terms of increased incidence*.

In conclusion, it is my recommendation that CARC consider the lines of reasoning offered here in deciding whether malathion should be designated a leukemogen.

References

Huff et al. (1985): Malathion and Malaoxon: Histopathology Reexamination of the National Cancer Institute's Carcinogenesis Studies, Env. Res. 37, 154-173.

National Cancer Institute (1979): Bioassay of Malathion for Possible Carcinogenicity. U.S. Department of Commerce, Technical Report Series No. 192

Office of Science and Technology Policy (1985): Chemical Carcinogens; A Review of the Science and Its Associated Principles. Fed. Reg. Vol. 50, No. 50, March 14, 1985, 10372-10442.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch/HED

Attachments (2)

ATTACHMENT 10

Mr. William Burnam May 18, 1999
Chairman
Cancer Assessment Review Committee
Health Effects Division

Re: May 3, 1999 report "Addendum to Malathion Qualitative Risk Assessment Based On Fischer 344 Rat Dietary Study", by Ms Lori Brunsman (copy attached)

In reading the referenced report on the statistical analysis of thyroid c-cell tumors, I have observations that I would like to convey to members of the Cancer Assessment Review Committee (CARC) for their consideration at the next CARC meeting.

You will notice on pages 3 and 4 of the referenced report, the finding of a statistically significant increase of thyroid c-cell carcinomas in Group 3. Also, when statistical treatment is confined to Groups 1-3, p. 4, there is a positive trend. It is difficult to say whether the decline in tumorigenic responses in Groups 4 and 5 were consequences of competing toxicity and/or decreased numbers of animals at risk due to high mortality, but I submit it is likely. Arguably, to the extent that carcinomas arise via progression of adenomas, fewer adenomas in a dose group, for whatever reason, diminishes the pool of adenomas within which carcinomas may arise. So competing toxicity and/or high mortality in male Groups 4 and 5 may have compromised expressions of c-cell carcinomas, whether such lesions arise de novo or by progression from adenomas.

In addition to the finding in Group 3, where by casual inspection one might conclude that only carcinomas as opposed to adenomas responded in any meaningful manner, there might be yet another perspective. I shall preface what I have planned to say by quoting from McConnell et al (1986), an authoratitive literature treatment on the interpretation of carcinogenesis studies. These investigators say: "A particular chemical that induces a statistically significant *shift* (emphasis added) in tumor expression from benign to malignant without the total incidence increasing may be regarded as a carcinogen. However, when the benign and malignant neoplasms are combined in such a case, the study would be classified as negative, implying that the chemical is noncarcinogenic. Thus combining neoplasms in this case would result in a false-negative effect." (p. 283) In the malathion study, the features of the data set for Groups 1-3 are characteristic of those in the quotation from McConnell et al, and should be evaluated accordingly. Though not evaluated in Lori's statistical analysis, there is evidence of a statistically significant shifting in tumor expression from benign to malignant with dosing, namely from one in fourteen in Group 1 to six in fourteen in Group 3, without an appreciable increase in combined tumor incidence. In my opinion this data set on thyroid tumors conforms well to the example presented in McConnell et al (1986), and constitutes additional reason to call the response positive. Stated differently, in consideration of the rationale of McConnell et al, one should not view the evidence of carcinogenicity in this data set as singularly that of an increased incidence of carcinomas, but in a more dynamic sense as deriving concertedly from adenoma *and* carcinoma responses, i.e. from the total tumorigenic response Hence, the CARC must avoid marginalizing the significant increase in carcinomas on the grounds that adenomas were not similarly increased, when in fact, taken together, the conditions of increased carcinomas *and* evidence of a shift in tumor

expression from benign to malignant, without the total incidence increasing, as described in McConnell et al, are met in this data. In my understanding of McConnell, this data embracing Groups 1-3, would be considered positive evidence of carcinogenicity.

I should also note as supporting evidence of a positive finding is tumor multiplicity in Group 3 as disclosed in Lori's assessment.

In evaluating the thyroid tumorigenic response among males in this study, excessive mortality in Groups 4 (74%) and 5 (100%) probably compromised the tumorigenic expression in the latter groups. In fact, there is some evidence in the data of a dose related decline in combined tumor expression in Groups 4 (16%) and 5 (11%) versus Group 1 (26%). So I must reiterate an opinion I have expressed previously to the committee, namely, to the extent this malathion combined toxicity/carcinogenicity study is considered negative for carcinogenicity among male rats, the study is unacceptable for the reasons in evidence with this tumor type. Furthermore, this opinion is not restricted to this tumor type.

Sincerely,

Brian Dementi, Ph.D., D.A.B.T.
Toxicology Branch 1
Health Effects Division

Attachment(s): one

Reference: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

ATTACHMENT 11

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

June 7, 1999

Re: Malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901), interstitial cell testicular tumors.

At earlier CARC meetings on malathion, when the subject of interstitial cell testicular tumor findings were under discussion, I was not satisfied with the opportunity accorded me to present the data. As the reviewing toxicologist, I consider it encumbent upon me to convey to the committee factual information pertinent to this issue while the matter is yet before us. At the last CARC meeting, 2/24/99, a committee member sought to discuss this tumor type further, but was discouraged in her efforts to do so. I realize it may be somewhat more time consuming, but nothing, within reason, should be considered beyond the point of reconsideration in our pursuit of proper decisions on an issue as important as the carcinogenicity of malathion.

At one of the previous CARC meetings, someone present expressed the opinion that interstitial cell testicular tumors are a very common and benign tumor type, in essence downplaying its significance as a tumorigenic response to be concerned over. In fact, Boorman et al (1990) say: *"Almost all interstitial cell neoplasms of the testis are benign: features of malignant interstitial cell neoplasms include invasion of the epididymis and rarely pulmonary metastases."* (p. 413). Admittedly, that claim did dampen, somewhat, my determination to speak to the issue.

In any case, in my recent review of the re-examination of nasal histopathology in the 2-year rat study, Dr. James Swenberg, author of the report cited a journal publication, McConnell et al (1986), as germane to his views on the interpretation of nasal tumor findings. However, this publication comments on a variety of tumor types, including testicular tumors. I should like to quote from that publication: *"For some neoplasms there is substantial evidence for the sequential progression from the hyperplastic to the benign stage and from the benign to the malignant stage. Progression has been suggested for the following lesions: epidermal skin lesions in mice, alveologenic lesions in mice, esophageal and forestomach lesions in rats, bladder urothelial lesions in rats, testicular interstitial cell lesions in rats, and prostatic lesions in rats."* (p. 284) So there is an authoritative claim that testicular tumors do progress to malignancy, so much so that this tumor type finds its place among a rather short list of tumors the authors cite in illustrating their point regarding progression. Perhaps a more in-depth review of progression of this tumor type should be pursued.

In the malathion study, interstitial testicular tumors were identified in the study submission itself (MRID 43942901) as a positive finding. Please find appended (attachment 1) a copy of the pages from the study report wherein the claim was rendered. Also, appended (attachment 2) is a copy of the statistician's report from the same source wherein the same claim is affirmed. Furthermore, it should be noted that HED's Lori Brunsman obtained essentially the same results, excepting the low dose group, in her statistical treatment of the data; see both her "Tumor Analysis" (p. 2) and Table

3 (p. 5) (attachment 3). In consideration of Ms Brunsman's findings, one will note that while incidences of this tumor type across the various dose groups was essentially the same, the statistically significant findings, both dose trend and pairwise comparisons, are remarkable. Interpreted, there was a dosing-related earlier onset (decreased latency) for this tumor type. Stated differently, tumor incidences in dose groups that were statistically significant were higher in dose groups than expected given the rats' shorter lifetimes. This evidence of dosing-related enhanced tumor development or decreased latency would appear to satisfy the Office of Science and Technology Policy (1985) characterization of a carcinogen, which reads as follows: "*A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen." (p. 10414-10415)*

The author of the malathion study report, as revealed in attachment 1, discounted the importance of this finding rationalized on the historical control data, while missing the point that incidences in dose groups were statistically higher than expected because of early mortality. In other words, such high incidences are normally late occurring, but in this study occurred earlier than expected in the treatment groups relative to the contemporaneous control group. This, of course, is as indicated above another example of dosing-related enhanced tumor development, which satisfies the OSTP definition of a carcinogen.

I experienced frustration at getting this across at the earlier CARC meetings, particularly in the absence of Dr. Hugh Pettigrew at one particular CARC meeting (10/8/97). However, after that meeting in discussing this issue with Dr. Pettigrew, he acknowledged to me in private conversation that the data supported a conclusion of decreased latency. (attachment 4). Also, at the subsequent CARC meeting (10/15/97), Dr. Pettigrew, according to my notes of the meeting, affirmed the study illustrates decreased latency.

I should emphasize before the committee that, as analyzed by the Peto test, this is a tumor type significantly increased in a dosing-related manner, embracing the 500 to 12000 ppm dose range. I should also note that when reviewing this topic, I discussed the matter with Dr. Joseph Haseman, NTP statistician, who recommended the Peto test as appropriate for this data. He advised, if performed properly, statistically significant findings would be valid. This gentleman even offered to perform the test for me, which I graciously declined. This finding assumes more importance than previously recognized by the CARC if this tumor does progress to a malignant stage as suggested in McConnell et al (1986). Indeed, it could be argued that with earlier onset, the opportunity for progression to malignancy within the individuals life time would be enhanced relative to controls.

My question to the CARC would be, did anyone at the CARC meetings refute this line of reasoning in discounting statistically significant increases in this tumor type as a treatment-related effect? If so, the committee should show cause, i.e. present the rationale for discounting the findings, rather than simply noting tumorigenic expression was the same in all groups, in spite of the statistics, and claiming incidences were within the historical ranges for control animals that survive two years.

References

Boorman, G.A., Chapin, R. E. And Mitsumori, K. (1990) Testis and Epididymis. Chapter 24, pp. 405-418. In Pathology of the Fischer Rat. Reference and Atlas. Academic Press, Inc. (Chapt. 24)

McConnell, E.E., Solleveld, H.A., Swenberg, J.A. and Boorman, G.A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. *JNCI*, 76, 283-289.

Office of Science and Technology Policy (1985) Chemical Carcinogens; a Review of the Science and Its Associated Principles. *Fed. Reg.* Vol. 50, No. 50, March 14, 1985, 10372-10442.

Brian Dementi, Ph.D., D.A.B.T.
Toxicologist
Health Effects Division

Attachments (4)

Pages 4-8 of this attachment have been claimed confidential. They are releasable to persons who submit a signed “Affirmation of Non-Multinational Status” form.

June 21, 1999 (2:20 PM) e-mail of Brian Dementi to William Burnam

William,

I would be pleased to respond to your memorandum of 6/18/99 to CARC members in the following way. You say in reference to the "Dosing Issues" section of the 1996 draft Cancer Risk Assessment Guidelines: "I don't think this section implies that significant changes in hematology, clinical chem., reduction of body weight gains etc cause the tumors, ..." I agree with this statement. In fact I believe it has not been demonstrated that cholinesterase inhibition was in any way responsible for tumorigenic findings in the rat or mouse studies, nor has it been demonstrated that cholinesterase inhibition was excessive in terms of undermining an MTD identified by other end point. You continue: "..., but they indicate that the results may be not suitable for risk extrapolation if the tumors are seen only in the presence of this frank toxicity." Yet, there is no evidence the cholinesterase inhibition observed in the rat or mouse studies was frankly toxic. The animals "tolerated" cholinesterase inhibition very well.

A general policy is needed for the use of cholinesterase data in the interpretation of carcinogenicity bioassays conducted with cholinesterase inhibitors. For the proper assessment of carcinogenicity, testing at doses up to and including an MTD should be pursued. It is quite possible that achievement of a true MTD on the basis of parameters other than cholinesterase inhibition may be precluded by cholinesterase inhibition for such compounds. In fact, because of this potential roadblock, cholinesterase inhibitors may receive kid glove testing for carcinogenicity relative to other classes of compounds. Where cancer bioassays are concerned, cholinesterase inhibition at a level considered excessive in the minds of some people, may not be viewed as toxic in terms of carcinogenicity testing if other evidence of toxicity does not exist, (e.g. cholinergic signs, unusual behavior, mortality, excessive body weight changes, etc.). In other words, it must be established by some rationale that cholinesterase inhibition per se, of a given magnitude, is to be considered toxic in such studies if other evidence an MTD has been exceeded does not exist, i.e. that inhibition of the enzyme, in and of itself, satisfies to identify an MTD, or its exceedance. I do no accept that such rationale exists. Who can say what degree of brain cholinesterase inhibition is to be considered excessive in the absence of any evidence of toxicity? If acetylcholine receptors have down regulated to accomodate cholinesterase inhibition, and animals tolerate the regimen just fine, where is the argument an MTD has been exceeded? Indeed such adaptation permits testing of cholinesterase inhibitors for carcinogenicity at proper high doses.

The draft 1996 Cancer Assessment Guidelines say the following: "Animal studies are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity of the tested agent. There is little doubt this may happen in some cases, but skepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al., 1993a; Melnick et al., 1993b; Barrett, 1993). In light of this question, *the default assumption is that effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized.* If adequate data demonstrate that the effects are <u>solely</u> (emphasis added) the result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be regarded as not appropriate to include in assessment of the potential for human

carcinogenicity of the agent." (p. 81) I am not aware that anyone has demonstrated at a CARC meeting, either on the rationale of cholinesterase inhibition or any other parameter of toxicity, that the tumorigenic findings in the malathion mouse study were "solely", or even primarily, the result of excessive toxicity rather than carcinogenicity of the tested agent per se."

I realize these comments come after the original data backage, but since they do respond to your comments submitted after that deadline, it would appear proper for you to forward these to the CARC before Wednesday's meeting.

Mr. William Burnam, Chairman July 13, 1999
Cancer Assessment Review Committee
Health Effects Division

At the June 23, 1999 meeting of the Cancer Assessment Review Committee (CARC) on malathion, when considering the findings of oral tissue squamous cell tumors, in an essentially *divided* vote, the committee discounted these tumors as related to treatment. I affirm that it remains until the final report of the meeting for a more precise rendering of the CARC's assessment of this tumor type. According to my notes, in contrast to CARC's vote on nasal tissue tumor findings, the committee discounted the oral tissue tumors on the grounds they are not sufficiently *rare* in the historical data base. This was based on the reading of historical control data from a 1998 NTP historical control data base on the F344 rat [Toxicology Data Management System: Tumor Incidence in Control Animals by Route and Vehicle of Administration F344/N Rats; Prepared for NIEHS by Analytical Sciences, Inc.,Duram, NC; OCR Services, Inc., Research Triangle Park; Feb. 1998] An assessment of that data was also discussed in Dr. Luke Brennecke's June 22, 1999 memorandum to Sanjivani Diwan, copies of which were provided by Dr. Brennecke at the June 23 meeting, and is also attached here. This data contrasted with historical data I had provided in my reviews, based on earlier publications of historical data. I was puzzled by the contrast, so following the meeting I obtained from Dr. Brennecke the bibliographic citation (rendered above) to the 1998 NTP data base, and obtained a copy from NIEHS. In reviewing this document for oral tissue squamous cell tumor incidences in F344 rats, I find it does not differ substantially from the 1996 version which was already in my possession. The main difference between the 1996 and 1998 versions is that the total number of animals in the data base declined from 1996 to 1998, as several studies performed in 1982-1984 were eliminated, while only a couple newer studies were introduced. Thus the number of male and female rats in the 1996 publication were 1354 and 1351**,** respectively, while in the 1998 version, the respective numbers were 904 and 901. In spite of the decline in total number of animals represented by the data base, the actual number of squamous cell tumors of the oral tissues changed ever so slightly. In my review and comments on the malathion study, I was citing yet an earlier version of the NTP data base [Haseman, et al (1990) Tumor Incidences in Fischer 344 Rats: NTP Historical Data. *In:Pathology of the Fischer Rat; Academic Press;* Chapt. 35, 555], as that was the very data base referred to in the combined chronic toxicity/carcinogenicity study on malathion as submitted to the Agency. I should note that Dr. Joseph Haseman remains the principal source of information for the 1998 version cited above.

In the malathion study report, the study director was citing incidences from the total data base, which included not only the oral feeding studies, but control data from other types of studies, e.g. gavage studies, as well. The number of animals from combined studies, at that time as reported in Haseman et al (1990), amounted to nearly 4000 rats of each sex, where in the case of the "Oral Mucosa (Any Site)", there were reported one squamous cell carcinoma and 7 squamous cell papillomas among nearly 4000 control males in untreated dietary and corn oil gavage studies. Similarly, among control females there were reported one squamous cell carcinoma and seven squamous cell papillomas among nearly 4000 control dietary and corn oil gavage studies. Six of the seven squamous cell papillomas in both males and females were identified among the nearly 2000 control corn oil gavage animals. Thus, since squamous cell tumors in question in the malathion study are of the oral cavity, untreated *dietary* historical controls as opposed to the

gavage dosed groups may be expected to be the more relevant. In which case for the dietary feeding studies the incidences for *both* males and females are one squamous cell carcinoma and one squamous cell papilloma of the oral mucosa from among nearly 2000 rats of each sex.

As I examine the 1998 NTP data base, now in my possession, my findings are at variance with Dr. Brennecke's findings as reported in his June 22 memorandum to Dr. Diwan. I presented my analysis to Dr. Brennecke in a memorandum of July 8, 1999, copy attached. I find that among female rats the incidence of squamous cell carcinoma of the *oral mucosa* (not counting tongue, pharynx, tooth, gingiva) in the 1998 NTP data base is 0/901, and in the 1996 NTP data base 0/1351. The respective incidences of squamous cell papilloma are 2/901 and 1/1351. Among male rats, respective incidences of squamous cell carcinoma of the oral mucosa are 1/904 and 0/1354 and for squamous cell papilloma 2/904 and 1/1354, respectively. This whole question of the NTP historical control data base is complicated as to interpretation by changes that have taken place during the very period of time the malathion study has been under review in HED.

These are clearly rare incidences in my estimation, regardless of which data base (i.e. which year) is cited. Furthermore, it is unknown by the CARC where, within all regions of the oral mucosa, these very few historical tumors were found, i.e. of the palate or elsewhere. In order for the historical incidence in the oral mucosa to be relevant to the malathion reevaluation, the entire oral mucosa would need to have been evaluated in the malathion study. Yet, only limited regions of the oral mucosa would be expected to have been evaluated in the *nasal tissue* reexamination before the committee.

In the NTP historical data base, squamous cell tumors of the *oral cavity* are presented as the sum of such incidences in the "oral mucosa", "tongue", "pharynx", "tooth" and "gingiva". A large fraction of *oral cavity* squamous cell tumors are of the tongue and pharynx, tissues not examined in the malathion study, either in the origional submission or in the nasal tissue reevaluation. Furthermore, only a portion of the total oral mucosa, perhaps the most relevant tissue in this case, was examined in the malathion *nasal tissue* reevaluation. It should be clear to everyone that the oral tissue findings in the malathion study are very rare when considering both the low incidence in the NTP data base for the oral mucosa and the limited portion of the oral mucosa evidently under review in the malathion nasal tissue reevaluation. *Indeed, the squamous cell tumors that were identified in so limited an assessment should serve as a signal that a proper assessment of the entire oral cavity be performed. Furthermore, until there has been a proper assessment, the squamous cell tumors already identified should not be discounted.*

This information should have been on the table prior to the June 23 meeting. Its absence prior to the meeting serves to underscore the need for everyone to have available critical information before hand. It should not be a requirement unique to the reviewing toxicologist to present information to the committee two-three weeks, or at least at some point in time, before the meeting. Furthermore, when a situtation such as this arises, where follow-up becomes necessary for whatever reason, there is an encumbency upon the committee to revisit the matter for proper evaluation, and another vote. It is also important that this information become a part of the record.

This letter should be distributed to all members of the CARC.

Brian Dementi, Ph.D., D.A.B.T.
Toxicologist

Attachments (2)

Pathology Associates International
A Company of Science Applications International Corporation

Rec 6/23/99 @ CARC Mtg

MEMORANDUM

SUBJECT: Review of the Histopathology Re-Assessment of Nasal Tissues for the Malathion 24-Month Oral (Dietary) Combined Toxicity/Carcinogenicity Study in the F344 Rat (MRID 4-4782301)

TO: Sanjivani Diwan
Reregistration Branch 4
Health Effects Division (7509C)

FROM: Lucas H. Brennecke, DVM, DACV P
Pathology Consultant
Health Effects Division (7509C)

DATE: 22 June 1999

Action Requested: Determine whether the peer review conformed with PR Notice 94-5, and determine whether the changes in pathology readings are meaningful.

I. Conformance with PR Notice 94-5

The report of the nasal tissue evaluation and peer review as well as Dr. Dementi's 27 May Memorandum (subject as above) were reviewed. As noted in Dr. Dementi's Memo, "the Agency was not seeking a Pathology Working Group (PWG) assessment. Rather, the Agency was seeking a Peer Review consisting of an initial assessment by the designated Study Pathologist followed by an assessment by a Reviewing Pathologist, and their concurrence or consensus." Therefore, although the review was not conducted in accordance with the provisions of PR Notice 94-5 with regard to a PWG review, it was reviewed in accordance with the Agency's wishes.

II. Adequacy of Sectioning

Five sections through each nose were to have been made in accordance with the techniques described in Eldridge, S.R., et al (1005), Fund. Appl. Toxicol. 27, 25-32. A total of 4,580 glass slides were prepared and reviewed. Judging from the diagnoses presented in the tables in the peer review document, the sectioning appears to be adequate, although the presence (or absence) of certain types of epithelium indicates that the sectioning may not have been completely accurate. For example, the adenoma in rat #5040 was thought to have originated in the respiratory epithelium in section 1. If sectioned accurately, however, section I (across the tip of the nose caudal to the external nares) should only include squamous epithelium.

III. Meaningfulness of Results

I completely agree with the report's author, Dr. Swenberg, that Malathion exposure was associated with clear evidence of nasal toxicity in the two highest exposure groups. The abundance of nasal toxicity and the patterns of distribution of the lesions noted in this dietary study underscore the fact that this chemical apparently aerosolized extensively. Because rats bury their noses in their feed (usually ground feed in a dietary study), this study was actually an inhalation study in addition to being a dietary study.

15 Worman's Mill Court, Suite I * Frederick, Maryland 21701 * (301) 663-1644 * (301) 663-8994 FAX

With regard to the neoplastic nasal lesions, it is my opinion that the findings reported in this peer review be accepted. Dr. Swenberg stated that there was final consensus on all of the neoplasm diagnoses. All of the tumors were diagnosed as well differentiated nasal adenomas. Although these tumors are rare, their numbers do not indicate a statistical significance. Dr. Swenburg stated that there was no common site. Their presence within the nasal cavity and their diagnosis as well differentiated nasal adenomas, however, indicate a common site (nasal mucosal epithelium). In addition, all of the nasal tumors were noted in Groups 4 and 5. The NTP historical controls show no incidences of nasal adenomas in control male or female F344 rats in inhalation studies, no control males in feed studies, and only one incidence of 1/50 female control rats in feed studies. The presence of even one male rat with such tumors is outside the range of controls, and the presence of even one female rat with such a tumor is at the limit of NTP controls. The tumors appear to be related to treatment, although it would be difficult to make a statistical case.

With regard to the neoplastic oral lesions, it is my opinion that the findings reported in this peer review be accepted. Dr. Swenberg stated that there was final consensus on all of the neoplasm diagnoses. All of the tumors apparently arose from squamous tissue within the oral cavity (whether it was the alveolus of the tooth or the epithelium lining the palate). Two of the tumors were in Group 2 animals (a squamous cell carcinoma in a Group 2 female and a squamous cell papilloma in a Group 2 male). The other two neoplasms were in Group 5 females (a squamous cell carcinoma and a squamous cell papilloma). The NTP has reported in its historical controls two incidences in which 1/50 female control F344 rats on feed studies have had squamous cell carcinomas of a tooth. The NTP historical controls also have one incidence of 2/50 female control F344 rats with squamous cell carcinomas of the oral mucosa (to include palate) and three incidences of 1/50 female control F344 rats with squamoUs cell carcinomas of the oral mucosa. Squamous papillomas are much more common, with five incidences of 2/50 or 3/50 female control F344 rats and six incidences of 1/50. In male F344 control rats, there were eight incidences of 1/50 or 2/50 papillomas. I believe that it would be difficult to make a case for the oral tumors to be considered treatment related.

ATTACHMENT 13

To: Dr. Luke Brennecke July 8, 1999

From: Dr. Brian Dementi

As we discussed by phone today, having obtained a copy of the February 1998 NIEHS report of historical control data in the F344 rat, I find my reading of the data is at variance with the reading presented in your memorandum of June 22, 1999 to Sanjivani Diwan. Before seeking a change in the record of the CARC meeting, I would be pleased to have your comments regarding my comparative assessment.

In the following paragraphs, your text is given in normal script, while my views are in bold italics.

"The NTP has reported in its historical controls two incidences in which 1/50 female control F344 rats on feed studies have had squamous cell carcinomas of a tooth." ***This represents 2/901 (0.22%) in females. In males there were 0/904 squamous cell carcinomas of the tooth. There were no reported findings of squamous cell papilloma of the tooth in males or females. (p. 307)*** "The NTP historical controls also have one incident of 2/50 female control F344 rats with squamous cell carcinomas of the oral mucosa (to include the palate) and three incidences of 1/50 female control F344 rats with squamous cell carcinoma of the oral mucosa." ***This amounts to 5/901 squamous cell carcinomas of the oral mucosa. However, independent inspection of the NTP historical control data base discloses in fact a 0/901 (0.00%) incidence of squamous cell carcinoma of the <u>oral mucosa</u> in F344 female rats. (p. 279)*** "Squamous cell papillomas ***(tissue site?)*** are much more common, with five incidences of 2/50 or 3/50 female control F344 rats and six incidences of 1/50." ***This would represent <u>at least</u> 17/901 (1.89%) squamous cell papillomas in female controls. Presumably the oral mucosa is being referred to here, as it was the tissue site referred to in the previous sentence on squamous cell carcinomas. However, again, independent inspection of the NTP historical data base discloses but two incidences of 1/50 or a total of but a 2/901 (0.22%) incidence of squamous cell pepilloma of the oral mucosa in female rats.(p. 279) It should be noted that for squamous cell papilloma, the incidence for <u>oral cavity</u> (including the sum of incidences of the oral mucosa, tongue, pharynx, tooth, gingiva) is 12/901 (p. 278), broken down as follows: oral mucosa (2/901) (p. 279), tongue (7/901) (p. 306), and pharynx (3/901) (p. 283).*** "In male F344 control rats, there were eight incidences of 1/50 or 2/50 papillomas ***(tissue site?)***." ***This would equal an overall incidence of <u>at least</u> 9/904. However, independent inspection of the NTP historical data base discloses but two incidences of 1/50, or an overall incidence of but 2/904 (0.22%), of squamous cell papilloma of the oral mucosa in F344 male rats. (p. 279) It should be noted that for squamous cell papilloma, the incidence for <u>oral cavity</u> (including the sum of incidences of the oral mucosa, tongue, pharynx, tooth, gingiva) is 9/904 (p. 278), broken down as follows: oral mucosa (2/904) (p. 279), tongue (5/904) (p. 306), pharynx (2/904) (p. 283). Furthermore, for male F344 rats the incidence for squamous cell carcinoma for the <u>oral cavity</u> was 1/904 (0.1%) (p. 278), which lesion was of the oral mucosa. (p. 279)***

In summary, among control F344 rats, the historical incidence of squamous cell tumors at issue here for tooth and oral mucosa are as follows:

Females: ***tooth: carcinoma 2/901 (0.22%), papilloma (none reported)***
oral mucosa: carcinoma 0/901 (0.0%), papilloma 2/901 (0.22%)

Males: ***tooth: carcinoma (none reported), papilloma (none reported)***
oral mucosa: carcinoma 1/904 (0.11%), papilloma 2/904 (0.22%)

ATTACHMENT 14

Mr. William Burnam, Chairman July 22, 1999
Cancer Assessment Review Committee
Health Effects Division

This is a follow-up to my July 13, 1999 memorandum to you concerning historical squamous cell tumor incidences of the oral mucosa in the F344 rat. Additional information has become available.

Dr. Brennecke responded to me by his memorandum of July 14. *I should note that he appears to affirm the correctness of my assessment of squamous cell tumors incidences of the various tissues of the oral cavity in the 1998 NTP historical data base.* Thus, according to the NTP data base, the incidences of squamous cell carcinomas and papillomas are as I related them to you June 13. Among female rats the incidences of squamous cell carcinoma and papilloma of the *oral mucosa* (which would include palate) are 0/901 and 2/901, respectively; and for male rats are 1/904 and 2/904 in the same respective order. I do not concur with Dr. Brennecke's view that *oral cavity* squamous cell tumor incidences should be considered relevant in assessing the rarity of such tumors of the oral mucosa, when many of the tissues of the oral cavity were not examined in the malathion study *nasal tissue* histopathology examinations. Such tumors of the tongue and pharynx make up the bulk of the historical incidences for the oral cavity, and neither the tongue nor pharynx was examined in this study. However, the presence of such tumors of the palate, given their rarity, underscores the need for a thorough oral cavity assessment.

We also need to recognize that the historical incidences of squamous cell tumors of the *oral mucosa* (which would include palate) represent a much more expansive area of the oral cavity than the area bounded by the palate, e.g. floor of the mouth, cheeks, etc. that, again, were not examined in the malathion study. As a matter of further interest, since the 1998 NTP data base does not say where, within the *oral mucosa,* the 5 squamous cell tumors (3 in males and 2 in females) identified above were located, I phoned Dr. Joseph Haseman at NTP, who pulled out the very studies in which these tumors were identified. Of the 5 control squamous cell tumors of the oral mucosa, 4 are described as "pharyngeal" , under the heading "oral mucosa". The remaining one is simply described as "oral mucosa" with no further localization. So four of the five relevant tumors of the oral mucosa are of the pharyngeal region, which Dr. Robert Maronpot, also of NTP, confirmed in conference with me on July 21 would not be seen in nasal tissue sections, unless perhaps the most posterior nasal tissue section were very oblique. Thus, of all 5 squamous cell tumors of the oral mucosa identified in the NTP control data base, only one *possibly* embraces the palate, and *none* of the palate have been actually reported.

Dr. Brennecke says in reference to my statement that there were no squamous cell papillomas of the tooth in the NTP historical data base is not germane since there were no papillomas in the malathion studies either. My response is that since we combine squamous cell carcinomas and papillomas, one should report historical incidences for both, not just the particular benign or malignant form actually identified in the study. The zero incidence of papillomas of "tooth"

serves to embellish the rarity of the squamous cell tumor type evident on the basis of the squamous cell carcinomas in the NTP data base. You and I know, all else being the same, had there been squamous cell papillomas reported for that site in the NTP data base, they would not have been ignored.

In summary:

1) As I had claimed in my reviews prior to the June 23 CARC, so it remains true upon subsequent close scrutiny of the NTP data base, that squamous cell tumors of the oral mucosa, particularly the palate, are extremely rare. Indeed, not one has been reported for the latter anatomic site, though the possibility exists (which I consider unlikely) that but one could be of that site among some 1806 male and female rats combined. Yet, in the malathion study, there are three of the palate.

2) The NTP data base shows for females an incidence of 2/901 for squamous cell carcinoma of the "tooth". I also requested that Dr.Haseman examine the NTP reports for these two tumors. Both of these are described as "gingival", under "tooth", and in *my opinion* would be relevant for comparison as to rarity with the one squamous cell carcinoma of the squamous epithelium of the alveolus of a tooth reported for a female rat in the malathion study. Obviously, the control incidence for that tumor of 2/904 remains very rare.

3) In consideration of the fact that the four nasal tumors were considered treatment-related while the four oral tumors were not at the June 23 CARC meeting, toward the end of the meeting a CARC member sought an explanation for this voting disparity. The CARC response was clear, the nasal tumors are very rare, historically, but the oral tumors are not. Yet, this subsequent and closer scrutiny of the NTP data base indicates that squamous cell tumors of the palate are as rare as the nasal tumors. The rationale for the difference in vote does not exist.

Given the closely divided vote at the CARC on this tumor type, I must reiterate the point made in my July 13 correspondence to you, that in view of this new and revised the matter should be re-visited by the CARC, and this memorandum should be circulated to all of its members for consideration. The fact remains, and is of concern to me that there are eight historically *very rare* tumors (oral and nasal, though of two different types) that have been identified in the nasal sectioning.

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist

Attachments (1)

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ATTACHMENT 15

Mr. William Burnam, Chairman
Cancer Assessment Review Committee
Health Effects Division

September 21, 1999

In reference to your August 26, 1999 memorandum to CARC members concerning my letters of July 13 and July 22, 1999, and that of Dr. Luke Brennecke of July 14, 1999 on NTP's historical control data for squamous cell tumors, I must express my disagreement with your opinion that "....regardless of the historical control values, the low non-dose related incidence of these tumors does not add to our previous weight of evidence decision." The problems I have with this assessment include: 1) The finding of a squamous cell *carcinoma* of the palate in a high dose group female and a squamous cell *papilloma* in a penultimate dose group female does constitute some evidence of a dose-related carcinogenic effect; 2) It is only proper for CARC to reach *correct* decisions on every tumor type, regardless of what it may be perceived to add to the weight of evidence; and 3) Were this tumor type positive, it *would* add to the weight of evidence, particularly if the carcinogenicity of malathion extends to the lower doses. I would be pleased to know whether any other CARC members have expressed any opinion, particularly with respect to the need to reconvene for another vote, in the light of the issues I have discussed in the previous memoranda.

Further to the point at hand, I cannot accept the notion that regardless of the historical rarity of a particular tumor type, low incidences of such a tumor can, or should be, discounted if not evidencing a treatment related effect. Indeed, low incidences of a very rare tumor type among the small number of animals employed in a cancer bioassay need not exhibit a treatment related effect to be viewed as of significant concern. I believe this is consistent with the opinions of experts, and is not, or should not be, so foreign to members of our Cancer Assessment Review Committee. Your's is one opinion, yet opininion was markedly divided on this topic at the June 23 CARC meeting, even in the face of incorrect historical control data information. As chairman and member of the CARC you have your vote, but you should not as chairman preempt a reconvening of the committee to consider the issue, now that more complete information on NTP's historical data is available.

I have been awaiting receipt of the draft CARC report since the committee's last meeting on June 23, to realize my opportunity to comment. Yet, in spite of the lack of finality of the CARC report, I find that the risk assessment for malathion is moving forward at a brisk pace. See the attached note of Paula Deschamp. In view of this activity, I consider I should wait no longer in expressing one of my major concerns over the malathion carcinogenicity data base, and I should note this has to do with the very contribution to the weight of the evidence the oral squamous cell tumor findings may offer. Namely, the collective evidence of a tumorigenic response at the lowest dose, 100/50 ppm in the combined chronic toxicity/carcinogenicity study in the F344 rat and 100 ppm in the carcinogenicity study in the B6C3F1 mouse study. The committee will recall in addition to the squamous cell tumors in the lowest dose group, the committee was presented with evidence that testicular tumors and leukemia (in terms of progression as evidence of carcinogenicity) in the male rat, and liver tumors in the male mouse extended to the lowest dose group. In addition, thyroid c-cell carcinoma among male rats was increased at the 500 ppm dose

level, a relatively low dose in comparison to the higher doses in the study of 6000 and 12000 ppm. Now I realize the committee also discounted the latter four tumor types, decisions *which I maintain should be considered by external peer review*. Nevertheless, these tumor types denote a low dose effect of the test material. Add to this evidence, the liver tumor response in female rats, which the committee affirmed as treatment related, and which also identified the 100/50 ppm finding as a tumorigenic response that cannot be discounted, there is substantial collective evidence of a tumorigenic response at the lowest dose, and there is no NOEL for tumorigenicity in these studies.

I should also note that other biological evidence of an effect of the test material at the lowest dose rests with cholinesterase inhibition seen at 100 ppm in the rat and mouse, which was statistically significant in the rat but not so in the mouse, and the evidence of liver gross pathology (masses) in the mouse study and nasal histopathology in the rat.

I anticipate presenting much more documentation in support of these concerns in my formal response to the CARC draft report, but since the risk assessment is upon us at this time, this issue needs to be raised here in this more timely fashion.

In the interest of the public health, HED should be much more concerned about low dose tumorgenic findings than those at high doses. Given the inherent weakness of cancer bioassays to assess tumorigenic effects at low doses, high doses, up to and including the MTD, are employed in animal cancer bioassays to enhance, or maximize, the potential to detect carcinogenicity. The disadvantages of high dose testing are recognized. However, when tumorigenic responses are *actually* seen at low doses, findings at high doses are relatively less important because they do not evaluate the test material near real human exposure levels.

Finally, where this memorandum is concerned, I question the usefulness of the Q* as computed for the female rat liver tumor response (July 13, 1999 memorandum of Lori Brunsman to Brian Dementi) to address real tumorigenic findings seen in that study at 100/50 ppm. I must say it strikes me that for a more insidious carcinogen, this approach may not adequately address risks at the lowest dose levels when actual tumorigenic responses are observed at such doses and for which there is no NOEL. *For this also I would desire the benefit of an external peer review.*

Please enable distribution of this memorandum to other members of the CARC.

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist

Attachments (1)

ATTACHMENT 15

MEMORANDUM

DATE: 7-14-99

TO: Brian Dimenti cc: Bill Burnam

FROM- Luke Brennecke /s/

SUBJECT: Response to your July 8 note (RE* NTP Historical Control Data)

I apologize for the delay in responding to your 7-8-99 note. I have been working off-site and was not able to gain access to the needed items in my office. Try as I might, I have been unable to find my copy of the 22 June Memorandum to Sanjivani Diwan (either in hard copy or on my computer). Nevertheless, since you quoted me from that memorandum, I will respond to the areas where you have noted differences.

1) With regard to the squamous cell carcinomas of the tooth, your summaries appear to be correct, although my statements were also correct. The observation that, "There were no reported findings of squamous cell papilloma of the tooth in males or females", while also correct, is not germane since there were no papillomas (or adamantinomas, or fibrosarcomas, or odontomas, etc.) in the Malathion studies either.

2) Regarding my statement about squamous cell carcinomas of the oral mucosa, I should have said oral cavity instead. If you substitute cavity for mucosa, the statement is completely correct. The point remains the same, however. As you pointed out during the meeting, the mucosal epithelium is the same for all of the tissues of the oral cavity (except the mature teeth), pharynx, esophagus, and even forestomach). Once again, your summary is correct, but, indeed, there is historical evidence of four studies in which the in6idence of squarnous cell carcinoma of the oral cavity is 2-4%.

3) With regard to the statement about papillomas in the oral cavity, I must apologize that I was looking at the category of squamous cell carcinomas and papillomas. The first point in that statement, however, is correct: Squamous papillomas (of the oral - cavity) are much More common. The table on p. 278 of the reference shows four studies in which there were 4% papillomas and an additional four studies in which there were 2% papillomas. Again, the important point is the commonality of the mucosa in the oral cavity.

4) With regard to the statement about squamous papillomas of the oral cavity, the statement should read as follows: In male rats there were seven studies in which the incidence of squamous papilloma of the oral cavity was 2-4%. Again, the important point is the commonality of the mucosa in the oral cavity.

Brian, pardon me for the small mis-statements in my earlier memo. The important point is that the lesions of the oral cavity in the Malathion study fall well within the range of the NTP historical controls.

ATTACHMENT 16

To: Jess Rowland from Brian Dementi 10/6//99

Comments on September 20, 1999 Draft CARC Report on Malathion
This is the typed version of my handwritten notations in my copy of the September 20 draft I had at the September 30 and October 4 meetings. This typed version is enhanced somewhat.

Comments in italics given by page.

vii - 1st paragraph: ...National Toxicology Program, *performed during 1978-1980.*

vii - 3d paragraph: ....to 50 ppm in both sexes from the 3 month time point for the duration of the study, *due to erythrocyte cholinesterase inhibition among females at 100 ppm.*

vii - 4th paragraph:....severe inhibition of cholinesterase activity *and increased mortality...*

vii - 5th paragraph: *You should mention nasal tumors among male rats also, even if the committee discounted these as evidence of carcinogenicity.*

viii - 2nd paragraph: *You should note, however, that in contrast to controls, several carcinomas were seen in dosed animals.*

viii - 4th paragraph: *Acknowledge committee has no explanation for the absence of a carcinogenic effect among females (combined incidence of adenomas and carcinomas: 2%, 0% and 4% at 0, 8000 and 16000 ppm, respectively) in the 1978 NCI study in contrast with the remarkable response at the same dose levels in the recent study among females (2%, 19% and 84% at 0, 8000 and 16000 ppm, respectively).*

viii - 6th paragraph:....(adenomas, 0.59% and carcinomas, 0.07%) *should be revised to 0.44% and 0%, respectively, based on the 1998 NTP data base. One should note that the NTP incidence is such that carcinomas in female rats are extremely rare, supporting the committee's conclusions that findings in the lower two dose groups cannot be discounted. There is no NOEL for liver tumors in females.*

ix - 1st paragraph: *Provide a brief statement of committee's rationale for discounting the dose levels eliciting important tumorigenic findings as excessive based on cholinesterase inhibition in the absence of clinical signs or other evidence dosing was excessive.*

ix - 3d paragraph:....in male rats at any dose level, *but expression may have been mitigated by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively.*

ix - 6th paragraph:....in one male rat at 12000 ppm, *and in one male rat (olfactory epithelium) at 6000 ppm.*

ix - 6th paragraph:....in the nasal region *(section 5) where little other histopathology was seen, in contrast to the extensive histopathology observed of the olfactory epithelium particularly in sections 2-4.*

ix - 6th paragraph: *Briefly say why the two nasal tumors in males were discounted.*

ix - last paragraph: *Leukemia finding at the highest dose in malaoxon study.*

x - 3d paragraph: *There is inconsistency in citing liver tumors in mice which you discounted and not mentioning nasal tumors in male rats which were likewise discounted.*

1- 4th paragraph: *Include something about those particular NCI carcinogenicity study findings that led the 1990 peer review to require further testing*

2 - Section B (i): *Show here or elsewhere in this section tumor findings for the 0, 8000 and 16000 ppm groups in the NCI B6C3F1 mouse study, and note as inexplicable the total absence of a liver tumor response in females as contrasted with the 84% incidence at 16000 ppm in the new study. This is important, and is needed to make the report more transparent. What are possible explanations? Test material used? Laboratory? Timing?*

3 - 1st paragraph: *Indicate that at 800 ppm, although not reportedly statistically significant at the $p \leq 0.05$ criterion, the incidence was more than 4-fold that of the control and undoubtably contributes to the highly statistically significant dose trend, $p = 0.000$*

3 - 3d paragraph:....but not at the mid dose (800 ppm) *(though still increased over 4-fold)* and...

4 - 1st paragraph:....at the 100 aor 800 ppm dose groups. *Increased incidences were observed at both 100 and 800 ppm, though neither was statistically significant. These increases undoubtedly contributed to the remarkable trend ($p = 0.000$). The 100 ppm group ($p = 0.075$) should not be so soundly discounted as evidence of carcinogenicity given the solid trend and higher incidences at the top two doses. Dose range is so enourmous in this study that testing at 0 and 100 ppm may constitute a different study from that at 0, 800, 8000 and 16000 ppm, i.e. there is no reason to accept that malathion would manifest its effects by one and the same mechanism across such a wide dosage range of 100 ppm to 16000 ppm..*

4 - Table 3, multiple tumors*: Multiplicy at 100 ppm consisted of livers with both an adenoma and a carcinoma (2) and liver(s) with two carcinomas (1, possibly 2 depending of how one large carcinoma attached to two liver lobes is interpreted); total examples of multiplicity: 3 certain, possibly 4.*

4 - last paragraph:....Dr. Brennecke, consulting pathologist, commented that *for statistical purposes (?)* In the evaluation....

4 - last paragraph: *Observation: multiplicity is clearly recognized among experts as a weighing factor in considering the progression or development of a tumorigenic response, which in turn is one of the principle defining characteristics of a carcinogen.*

5 - 1st paragraph:....statistical significance at any dose level. *They are there nonetheless.* In addition....

5 - 2nd paragraph:....and 3 mice in one study (6.4%). *The historical data base from the performing laboratory is too small to be of much value in the interpretation. This should mandate placing greater reliance upon the much more relevant contemporanous control. Furthermore, it should be noted there is no backup historical control data from NTP, as that data base consists of full two-year studies in B6C3Fi mice.*

5 - 3d paragraph: *Since this is a summary paragraph, what is to be said of males at 100 and 800 ppm? In summary, this section of the report does not present the rationale for discounting the findings in the low dose group as presented in the December 1, 1998 signed review of the PWG report on mouse liver tumors (males). Nor does this cited review find its citation in the Bibliography (Section IX, pp. 28-29)*

8 - 1st paragraph: *Include incidences of macroscopic liver "masses" in all groups.*

8 - last paragraph: *Indicate why the study was performed at these high dose levels. Specifically, the registrant was requested to use these doses as they are the same as those used in the earlier NCI study. The study was required by EPA to address "equivocal" hepatocellular tumorigenic findings in <u>males</u> only in the NCI study. The limit dose is actually very arbitrarily set, considering the FIFRA Guidelines earlier set it at 5% of the diet. Also, 8000 and 16000 ppm are not that far above the current limit dose, particularly in view of how arbitrarily it was established. Also rationale should be provided for concluding that 800 ppm is an adequate and legitimate high dose to evaluate carcinogenicity potential since it is quite below the limit dose, and given the findings at the higher doses that have been discounted.*

9 - 3d paragraph: There was (were) no statistically significant increases in hepatocellular tumors at any dose level in male rats. *However, excessive mortality and/or competing toxicity may have precluded detecting a tumorigenic response at 6000 ppm (74% mortality) and at 12000 ppm (100% mortality). The principle of concern is most clearly llustrated in this very study in the case of leukemia incidence, where among 55 male rats per group, the number diagnosed with leukemia were 23, 16, 24, 18 and 1 for the 0, 100/50, 500, 6000 and 12000 ppm groups, respectively. Leukemia expression was almost precluded at 12000 ppm, and may have been compromised at 6000 ppm. Given that concern, and absent a satisfactory dosing level above 500 ppm, say in the neighborhood of 2000 ppm, compromises the capability of this study to assess reliably responses in male rats. This concern resides not only with liver carcinogenicity assessment in males. The F344 rat may be a poor model for malathion carcinogenicity assessments in male rats.* As shown in Table 6, in female rats.....

9 - Table 6: *It is noteworthy that for carcinomas the trend test (p = 0.063) is at the waters edge of statistical significance and beggs some comment, particularly since this is an exceedingly rare tumor type according to the NTP data base. A note is being introduced into the record showing that the female hepatocellular tumor responses in the latest (1998) NTP data base for oral feeding studies are: adenomas: 4/901; carcinomas: 0/901: and for all study types: adenomas: 18/3033; carcinomas: 0/3033.*

10 - 4th paragraph: *Should say this was a <u>nasal</u> tissue reevaluation, and oral tissue findings were incidental in the nasal tissue assessment, which reflects only a partial histopathologic assessment of oral cavity tissues.*

10 - table: *Initial heading should be males, which covers the first three rows of data, while females cover the last four rows of data.*

11 - 1st paragraph: *As to the reported NTP historical tumor incidence for the "respiratory tract" of 6/4000 for males, all six were of the respiratory epithelium. Hence, the incidence for tumors of the olfactory epithelium is 0/4000 controls. Furthermore, of the 6/4000 of the respiratory epithelium, four of the six were squamous cell tumors. The relevant incidence for the tumor type in question is therefore 2/4000 control males. See the DER for MRID 44782301, pp. 14-15.*

11 - 2nd paragraph:....2) all four tumors occurred in the nasal region of the two exposure groups exhibiting considerable nasal toxicity (hyperplasia of the olfactory epithelium); *Actually, as discussed on p. 19 of the DER (MRID 44782301), the two nasal tumors in females were found in nasalturbinate sections 5, which were not examined in the origional study, and is a region in which little other histopathology was observed. Thus the implication these tumors are secondary to other toxicologic effects is weak in these cases.*

11 - 2nd paragraph:....the incidences exceeded the *zero* historical control incidence.....

12 - 1st paragraph: *Suggested additional sentence: "In consideration of the increased incidence of carcinomas at 500 ppm and at 6000 ppm, and considering the excessive mortality (and possible competing toxicity) among males at 6000 ppm (74%) and at 12000 ppm (100%), the findings suggest a more remarkable effect would have been observed at say 1000-2000 ppm, a dose range not evaluated. Hence, to the extent that the finding at 500 ppm is discounted, it serves to underscore the inadequacy of the study."*

12 - Table 9: *In Lori Brunsman's May 3, 1999 statistical analysis report on this tumor type, two tables were provided. The second of her tables (absent dose groups 6000 ppm and 12000 ppm, since these dose groups for males were discounted by the committee) should also be provided here, as that table clearly shows a positive dose trend and pairwise comparison for carcinoma at 500 ppm, a dose level considered acceptable and adequate by this committee.*

13 - 1st paragraph: This conclusion was based on lack of statistical or biological significance at any dose level, there was no dose-response, and the incidence were within the historical control incidences of the testing laboratory (6/239 (20.8% for adenomas and 6/239 correction needed here (2.5% for carcinomas). *To the contrary, there was statistical significance for increased carcinoma at 500 ppm, whether or not the top two dose groups are included in the analysis. Also, when the top two dose groups are excluded as excessive doses, the trend also is positive. See Lori Brunsman's May 3, 1999 memorandum. The incidence of carcinoma at 500 ppm is not within the historical control range. There is no mention of the importance of tumor multiplicity in the 500 ppm dose group. Furthermore, the claim cannot be made here as was made for thyroid follicular cell tumors among males on p. 12, 1st paragraph, namely:* "This conclusion was based on the fact that when the two excessive toxic doses (6000 and 12000 ppm) are excluded, there is no increase in the incidence of adenomas, carcinomas or the combined adenomas/carcinomas in treated animals when compared to controls." *This argument should have equivalent applicability in both cases. An argument used in one case to discount findings, is ignored in another similar case when it supports a positive finding. This argument should have equivalent applicability in both case, and is particularly important in the thyroid c-cell case, as Ms Brunsman reported the statistics both ways, and because the finding was positive for carcinoma.*

The data also suggest that given the 6000 ppm and 12000 ppm doses were excessive, a more remarkable effect would be observed at say 1000-2000 ppm, a dose level not tested, an observation that underscores the inadequacy of the study if not called positive at 500 ppm.

13 - 1st paragraph: *There is no reference here or in the Bibliography to Dr. Dementi's May 18, 1999 memorandum to the CARC Chairman (Exhibit J10 for the June 23, 1999 CARC meeting) presenting certain additional important discussion of the c-cell tumorigenic response in males.*
15 - 2nd paragraph: *There should be some reference to the fact that the statistician's report in the origional study submission (MRID 43942901) (p. 5345-5346) (p. 157-158 in June 23, 1999 CARC package) concluded*

that increases in testicular tumors were statistically significant at all doses. Also, in the presentation of this tumor type there is no mention here, or reference in the Bibliography to Dr. Dementi's June 7, 1999 memorandum (Exhibit J11 in the June 23, 1999 package) to the CARC Chairman.

15 - last paragraph: *There is no reference here or in the Bibliogyaphy to Dr. Dementi's April 27, 1999 memorandum (J9) to the CARC Chairman on increased mortality (dosing-related) due to leukemia among animals with leukemia as evidence of carcinogenicity among male rats by OSTP's (1985) second aspect (decreased time of tumor development) of its definition of a carcinogen.*

16 - table 16: *While it may be space saving to consolidate the total findings in all five nasal turbinate sections, yet tumors in females occurred in nasal turbinate section 5 in both cases, where unlike sections 2-4, little remarkable dosing-related histopathology occurred, particularly at the top two doses where the tumors were seen. The data as tabulated obscures this observation. Therefore, it should be acknowledged in the text.*

Also need to acknowledge in some manner the fact that characterization of the effects of malathion on nasal tissues lacks completeness, as evidenced by no NOEL's for nasal tissue histopathology in the subchronic inhalation study (HIARC is requiring another study), the 2-week dose range-finding inhalation study, and the surprising nasal histopathology seen in the mouse 18-month carcinogenicity feeding study (wherein a NOEL was identified) and in this combined chronic toxicity/carcinogenicity feeding study in the rat which did not identify a NOEL for histopathology among females.

17 - 1st paragraph: *Reiterate disagreement, for example, with the committee's view that nasal tumors seen in males at 6000 ppm and 12000 ppm are to be discounted because these dose levels were excessive, while discounting c-cell carcinoma increases among males at 500 ppm with the argument such increases were not seen at 6000 or 12000 ppm. This represents a dual standard with respect to use of findings at doses considered as excessive, that works in a counter-conservative way where protection of the public health is concerned.*

19 - 4th paragraph:....was within the historical control range. *This is not sufficient reason to discount the finding.*

19 - last paragraph: *The brain cholinesterase data appears to be incorrect (see DER for MRID 43975201, p. 15). Also, the magnitude of inhibition of cholinesterase in the malaoxon sudy at 1000 and 2000 ppm is such that I see inadequate differentiation between inhibition in this study and the malathion study to justify not discounting the 1000 ppm dose as excessive for malaoxon.*
This problem serves to illustrate the arbitrary use of cholinesterase data by the committee to decide which dose levels are excessive, and underscores the need for precident and guidelines pertaining to this subject.

22 - 1st paragraph: *Is 50 ppm equivalent to 4 mg/kg/day?*

22 - 2nd paragraph: *Should mention HIARC requirement of another inhalation study.*

22 - last paragraph: *Certainly one of the major chronic toxicity findings was that of nasal tissue histopathology, where as the outcome of the histopathology reevaluation, a NOAEL was not identified for females. A NOAEL of 100/50 ppm (the lowest dose) was identified in males. For confirmation see the May*

27, 1999 DER for MRID 44782301.

24 - 3d paragraph: No statistically significant increases in carcinomas alone was seen at any dose level, *though there were numerical increases.*

24 - 4th paragraph:....was slightly out side the historical control range, *and well above the mean value in a small historical data base. Unfortunately, NTP's data base is for full two year studies, and cannot be used in this comparison.*

25 - 2nd paragraph: *Revise to show 1998 NTP historical control data for adenomas and carcinomas, 0.44% and 0%, respectively, for oral feeding studies.*

25 - 3d paragraph: No *statistically significant increase in* liver tumors occur at the 800 ppm dose.....

25 - 5th paragraph: There was no evidence of *liver* carcinogenicity in male rats at any dose level.

25 - last paragraph: *As noted on my comments for p. 11, of the 6/4000 historical incidence, four were squamous cell tumors, while 2/4000 were of the relevant tumor type (adenomas) in males. It is also noteworthy that of the 4000 historical controls, half were oral feeding and half corn oil gavage. The two adenomas were among the nearly 2000 corn oil gavage fraction.*

26 - 2nd paragraph: *Should say something about the committee's consideration of the oral cavity squamous cell tumors.*

26 - 3d paragraph:....and the tumors of the palate in male *and female* rats,.....

27 - 1st full paragraph: *Suggest removing* "but at the low end of this category", *as its meaning is not explained and I cannot recall hearing it spoken.*

Bibliography

As mentioned in these comments, the following should be introduced to the Bibliography: DER for the male mouse liver tumor PWG report; J9; J10; and J11.

The following memoranda of Dr. Dementi to the CARC Chairman should also be added to the Bibliography for completeness of the historical record and for any future reference interest:
memorandum of November 26, 1997 (copy appended to J9), and memoranda this year dated June 21, July 13, July 22 and September 21. The April 1, 1999 (J14) comments in response to the March 24, 1999 draft CARC report. Also the memorandum of June 16, 1999 to Ms Marcia Mulkey. If this latter memorandum was not forwarded to the committee, I will provide a copy.
I trust the CARC Chairman's August 26, 1999 memorandum will be included. Of course these present comments of 10/6/99 would be included as well.

ATTACHMENT 17

To: Jess Rowland from Brian Dementi October 28, 1999

Comments on October 28, 1999 Draft CARC Report on Malathion

Please find appended the most recent CARC draft report on malathion, dated October 28, 1999 (received around October 21), with my comments presented in the margins. In commenting (10/6/99) on the previous draft dated September 20, I provided you a *typed* set of comments, which I would prefer to provide in this case, but in the interest of time I have elected to forward comments as they appear in the margins. If you have trouble deciphering my penned comments or if you would prefer a set of typed comments, then please advise accordingly.

As you know, I have a number of differing views with respect to conclusions of the committee, and how conclusions should be framed in the text. Concerning this CARC document, I have presented by observations and opinions in my letter to you of October 6, and again now at this time in response to the more recent version. It remains to be seen just how much departure remains when the final report is signed. On seeing that report, it is likely that I will require the opportunity to submit a final written dissenting opinion on all outstanding issues.

I am concerned over what appears to be a dichotomous use of the two male high dose group findings in the malathion F344 rat study. The committee has said that in the case of male rats, 500 ppm is an acceptable dose, while the 6000 ppm and 12000 ppm doses are excessive. In making this distinction, the evaluation of tumorigenic findings has thus become subject to abuse, as is evident when the committee adopts the view that a tumorigenic response seen at the high doses can be discounted (nasal tumors, follicular cell thyroid tumors), while negative tumorigenic findings at high doses can be affirmed in discounting positive findings at lower doses (thyroid c-cell carcinoma). In fact, in your September 20, 1999 draft, you claimed in the case of follicular cell tumors: "This conclusion was based on the fact that when the two excessive toxic doses (6000 and 12000 ppm) are excluded, there is no increase in the incidence of adenomas, carcinomas or the combined adenomas/carcinomas in treated animals when compared to controls." (p. 12) To the extent this rationale can be put forward, then one might claim in the case of thyroid c-cell carcinoma: *The conclusion that the thyroid c-cell carcinoma incidence was increased at 500 ppm ($p = 0.013$), with a positive trend ($p = 0.006$), was accepted as positive evidence of carcinogenicity when the two excessive toxic doses (6000 and 12000 ppm) were excluded.* Now obviously when one declares a dose more than ten-fold higher as excessive, and then proceeds to pick and choose when to employ its findings, the study is to that extent flawed and unacceptable. The interpretation of findings at the "excessive" doses becomes a no win or no lose situtation, depending on how one wishes to employ the data. According to my understanding, accepting tumorigenic findings at excessive doses is more defensible than accepting as negative a study without findings at excessive doses. (See my Nov. 26, 1997 memo to Burnam)

In my opinion, in the case of pesticides with widespread use on foods, the Agency should be particularly concerned about tumorigenic findings at low doses. The philosophy behind high dose testing is to maximize the capability of detecting tumorigenic potential that may be difficult to identify at lower doses in an animal bioassay involving essentially small numbers of animals. So when a clearly statistically significant tumorigenic response is seen in the low dose range, indeed a range considered acceptable, one should think long before discounting such findings rationalized on the basis of findings at a dose more than ten-fold higher, which is

viewed as excessive, and at which dose level full expression of the tumorigenic response may have been compromised by competing toxicity and/or increased mortality. At the very least, this study should be affirmed as positive for c-cell thyroid carcinoma, or declared unacceptable. In this particular case, a dose increase from 500 ppm to 6000 ppm is too broad to properly evaluate the thyroid c-cell tumorigenic response in the 500 to 6000 ppm dose range. The rationale that thyroid c-cell carcinoma should be considered as positive since it occurred in the dose range considered adequate by the committee, is supported by the committee's affirmation of the importance of the liver tumorigenic response in females as having been observed in the dose range considered as adequate by the committee. "The most compelling evidence of carcinogenicity was shown in the response of liver tumors in female rats at doses which were not considered excessively toxic." (p. 31 of the present draft). Now in this case I realize the committee cited the more pronounced tumorigenic response at 12000 ppm (considered excessive in females) in support of the liver tumor finding, but the finding at 12000 ppm was positive, and no evidence exists that the tumorigenic response was solely the result of toxicity rather than carcinogenicity of the test material, that would support discounting the finding. Also, the dose was only twice that of the highest acceptable dose.

In my recent discussion with you on my October 6 comments, you indicated certain of these would be discussed with Bill Burnam prior to making the suggested changes in the draft. Please let me have the benefit of those opinions.

ATTACHMENT 18

Brian Dementi
11/12/99 02:42 PM

To:
William Burnam/DC/USEPA/US@EPA
cc:

Subject::Response to your Nov 5 note to CARC concerning my note of Oct 28 to Jess Rowland

I appreciate your November 5 memorandum to CARC members responding to my comments of October 28 to Jess Rowland. This is a very important subject that merits debate. Accordingly, I have a few more ideas to put forward for the CARC's consideration.

I don't believe the committee has had the opportunity to read my actual comments of October 28, most likely because these were submitted in hard copy along with my other suggested changes penned in the margins of the CARC draft report. In order for the CARC members to fully appreciate both your comments of November 5 and my comments of October 28, the committee should be availed of both. To that end, I am sending a copy by another e-mail memo to you in the hope you will distribute it as well as this current memo to the committee.

Concerning your comments of November 5, the quote from the draft Agency Guidelines actually does not settle the matter. There is too little guidance in the draft Guidelines pertaining to the question at hand. For example, under *Excessive high does* ,item b) says: *"Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits"*. This statement is woefully inadequate in providing direction. In the malathion F344 rat study in question, mortality was excessive at both the 6000 ppm and 12000 ppm doses in males, which instructs that dosing was excessive, at least to the extent that increases in mortality were attributable to effects other than cancer (see top p. 2-11 of the said Guidelines). Now you have indicated in your November 5 correspondence that: *"Again, even though the two highest doses were considered excessive, there were sufficient rats at risk to be used in a carcinogenic analysis by Peto's Prevalence Test."* While this might be true, incresed mortality and/or competing toxicity (chronic nephropathy in this particular case) likely precluded tumor expression. This point is illustrated most clearly in the case of leukemia incidence among male rats. Leukemia incidences among 55 rats per group were: 23, 16, 24, 18 and 1 at the respective doses of 0, 100/50, 500, 6000 and 12000 ppm. This data indicates that leukemia expression was actually precluded at the 12000 ppm dose, and consequently this dose level should not be considered relevant in assessing leukemogenic potential in terms of incidence at the lower doses. It could also be reasonably speculated that leukemia incidence at 6000 ppm was eroded by competing toxicity and/or elevated mortality (74%), and therefore, though the effect is less dramatic than at 12000 ppm, findings at 6000 ppm should also be considered unreliable in evaluating leukemia incidence at 6000 ppm, or in interpreting findings at doses below 6000 ppm. I use the example of leukemia because at the 12000 ppm dose level it best illustrates the point I seek to make. However, to varying degrees all other tumor findings observed at the two excessive dose levels, may be under represented because of competing toxicity and/or increased mortality. Every tumor type has its own story, but as with leukemia, thyroid c-cell carcinoma expression may have been similarly compromised in males at 6000 ppm and 12000 ppm where mortality was 74% and 100%, respectively, and hence findings at the top two doses cannot be used with reliance in rendering a decision as to what occurred at the lower doses, those doses considered acceptable by the committee. The Guidelines you cite do not address this particular situtation, but suggest that reason should prevail. Guidance in this case needs to be sought from other sources of information.

The Guidelines say: *"The default assumption is that effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized. Animal studies are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose). Consequently, the question often arises whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption, rather than inherent carcinogenicity of the tested agent. There is little doubt that this may happen in some cases, but shepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al., 1993a; Melnick et al., 1993b; Barrett, 1993). If adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent per se, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent."* Now I am not aware the CARC determined that nasal tumors or thyroid follicular cell tumors in males in the malathion study, or leukemia at the high dose level among males in the malaoxon rat study, or liver tumors at the top two dose levels in the mouse study were "*solely the result of excessive toxicity rather than carcinogenicity of the tested agent per se."* This quotation from p 1-12 of the draft Guidelines actually seems to argue in favor of accepting positive findings at excessive doses, unless clearly contraindicated, and is consistent with **not** accepting as negative, findings that come up that way at excessive dose levels.

This concept is supported by other authoritative sources. For example: *"Positive results obtained in one species only are considered evidence of carcinogenicity.* ***Positive results*** (emphasis added) *in more limited tests (e.g., when the observation period is considerably less than the animal's lifetime), but by experimentally adequate procedures, are acceptable as evidence of carcinogenicity.* ***Negative results*** (emphasis added), *on the other hand, are not considered evidence of lack of a carcinogenic effect, for operational purposes, unless minimum requirements have been met."* [Interagency Regulatory Liaison Group (IRLG) (1979), p. 248]; *"It is generally recommended that more than one dose level be tested. Most carcinogenic effects show a positive dose-response relationship, but maximum tumor incidence in test animals may not occur at the highest dose* ***when competing toxicity prevails*** (emphasis added). " [IRLG (1979), p. 250]; "*It is important to estimate the highest dose level that will be tolerated by the test animals during lifetime administration, i.e., the estimated maximum tolerated dose (EMTD). The EMTD is defined as the highest dose that can be administered to the test animals for their lifetime and that is estimated not to produce a) clinical signs of toxicity or pathologic lesions other than those related to a neoplastic response, but which may interfere with the neoplastic response, b) alteration of the normal longevity of the animals from toxic effects other than carcinogenesis; and c) more than a relatively small percent inhibition of normal weight gain (not to exceed 10%). The EMTD is determined on the basis of prechronic tests and other relevant information. If the test reveals that the EMTD is too high to meet the conditions defined herein****, positive results*** (emphasis added)*obtained above the EMTD are acceptable as evidence of carcinogenicity unless there is convincing evidence to the contrary. Alternatively,* ***negative result****s*(emphasis added) *obtained above the EMTD are considered inadequate unless particularly strong and specific scientific reasons justify their acceptance as negative.* ***Positive results*** (emphasis added)*obtained at or below the EMTD provide evidence of carcinogenicity."* [IRLG (1979), p. 251]

"The best negative evidence for the carcinogenicity of a substance is obtained from tests in which both exposure and observation last through all or nearly all of the expected life-spans of the animals under study. Negative results decrease in value as the exposure and observation periods are shortened, and they become practically meaningless if these periods are shorter than half the life-spans of the animals. When some animals die early in the course of a test, the value of the test is reduced as a function of the percentage of animals dying without tumors at periods markedly shorter than the life-span of the species. Sometimes, a positive carcinogenic response may be definitely demonstrated in a shorter period of observation if the experiment is adequately controlled; in such cases the test is considered valid if it is shorter than usual."

[IRLG (1979), p. 251]

"However, overtly toxic test doses may be of little value in quantitatively assessing human risk from low level exposure if there are differences in metabolism, pharmacokinetics or detoxification at various exposure levels. Furthermore, the target cells of a potential carcinogenic effect may be killed, since carcinogens are generally toxic to their target cells at sufficiently high doses. This, or early death of the test animals themselves, may actually ***mask*** (emphasis added) *a positive effect. Although minimal toxicity should be induced at the high test dose where feasible, overt toxicity or organ dysfunction* (note for example in the malathion study, nasal respiratory epithelium was adversely affected and the olfactory epithelium was highly compromised if not totally ablated in males at the 6000 ppm and 12000 ppm dose levels) *should be avoided."* [Food and Cosmetic Toxicology (1978); 16(suppl. 2), p. 101]

"A ***negative***(emphasis added) *study is ordinarily accepted by regulatory agencies if: .....survival of all groups (per sex per dose) is no less than 50%.....at 104 weeks for rats."* [Office of Science and Technology Policy (1985), Fed. Reg. Vol. 50, No. 50, p. 10414]

In summary, I have endeavored to present authoritative sources indicating that one cannot rely upon negative findings at doses considered excessive to conclude a test material is negative for carcinogenicity, and like unto this, one cannot use such negative findings at excessive doses in a particular study to discount positive findings at lower doses considered to be in an acceptable dose range. However, one can accept as positive evidence of carcinogenicity findings at excessive doses, except when according to the Guidelines *".... the effects are* ***solely***(emphasis added) *the result of excessive toxicity rather than carcinogenicity of the tested agent per se,....."*
"..

The referenced material is particularly germane to the interpretation of thyroid c-cell tumorigenic response among males in the 0-500 ppm range in the recent malathion study, and serves to underscore the statement made in my October 28 memorandum, namely: "*According to my understanding, accepting tumorigenic findings at excessive doses is more defensible than accepting as negative a study without findings at excessive doses"*

A couple other items in your November 5 letter I would like to comment on include: 1) in reference to liver tumors in mice, you say there was no increase in either sex at the lower doses. Actually, increases were seen in males (accompanied by multiplicity at the lowest dose) in the lower doses, but they did not achieve statistical significance at the P= 0.05 criterion; 2) concerning the same study you say the two high dose groups were judged by the CARC to be excessive. One of the reasons for that judgement as I recall was the doses were said to exceed the limit dose per the Guidelines. Yet I find on p. 2-11 of the draft Guidelines that the limit dose for feeding studies is 5% of the test material in the diet, while the 1000 mg/kg limit dose is applicable to oral gavage studies. To my understanding, the high doses in the mouse feeding study, 8000 ppm and 16000 ppm, did not exceed the limit dose. Hence, the CARC report needs to be revised in this respect; 3) you say *"The combined incidence of adenomas and carcinomas of the male thyroid follicular cell showed a significant trend but no increases by pairwise analysis"* This fact goes to the heart of the matter. Though a statistically significant trend existed and the increase seen at 6000 ppm nearly achieved statistical significance, we must be concerned in our interpretation that competing toxicity and/or increased mortality in the high dose groups may have precluded full expression of the response in these groups. The data denote a finding that cannot be refuted, because the study is compromised at the high doses as acknowledged by the CARC.

ATTACHMENT 19

Mr. William Burnam
Cancer Assessment Review Committee
Health Effects Division

December 7, 1999

Please find appended a copy of a November 18, 1999 memorandum to Patricia Moe (SRRD) and Paula Deschamp (HED) conveying the results of correspondence betweed myself and Dr. Henry Bolte, study pathologist for the malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901). I had mentioned the subjects at the more recent CARC meetings, but explained the action remained to be completed. Even though this report comes after what may have been the last CARC meeting, I believe it should constitute a part of the CARC record on malathion. This is a copy of the file copy, so the attachments are included for your inspection.

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist

Attachments (1)

MEMORANDUM November 18, 1999

SUBJECT: Malathion: Study Pathologist's Responses (MRIDs 44837001; 44970601; 44970501) to Health Effects Division's Questions on the Nasal Tissue Histopathology Re-evaluation (MRID 44782301) of the Combined Chronic Toxicity/Carcinogenicity Study in the F344 Rat (MRID 43942901).

FROM: Brian Dementi, Ph.D., D.A.B.T.
Toxicology Branch I
Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D.
Senior Branch Scientist
Toxicology Branch I
Health Effects Division (7509C)

TO: Patricia Moe
PM Team 53
Special Review and Reregistration Division (7508W)

TO: Paula Deschamp
Risk Assessor
Reregistration Branch 2
Health Effects Division (7509C)

Registrant:	Cheminova Agro A/S	Submission Nos:	S569531; S569540; S569558
Chemical:	Malathion	P.C.Code:	057701
Case No.:	818961		
DP Barcode:	D260115; D260116; D260120		
MRID Nos:	44837001; 44970601; 44970501		

In the process of reviewing the histopathology re-examination of nasal tissues (MRID 44782301) submitted by the registrant in response to HED's Cancer Assessment Review Committee's request for such re-examination, further information was sought from the registrant. Specifically, it was noted that in the original study submission of the two-year study (MRID 43942901), the diagnosis for male rat #5040 from the high dose study group as identified in nasal/turbinate section 2 was "nasal mucosa (olfactory): carcinoma" (p. 4100)(copy appended); however, for this same slide, the diagnosis by the same study pathologist, Dr. Henry Bolte, as submitted to Dr. James Swenberg in the re-examination (MRID 44782301) was "nasal mucosa (respiratory): epithelium-hyperplasia, multi-focal, slight" (p. B-1191)(copy appended), while Dr. Bolte's diagnosis for nasal/turbinate section 1 (a new slide not

obtained in the original study) was "nasal mucosa (respiratory): adenoma" (p. B-1190) So for this rat, it remained that a neoplasm was identified in the nasal turbinates, but the character of that neoplasm was revised by the Study Pathologist from carcinoma of the olfactory epithelium in section 2, to that of adenoma of the respiratory epithelium of section 1, with no tumor finding in section 2. When the reviewing pathologist, Dr. Swenberg, examined section 2, he identified an adenoma, which the two pathologists agreed extended from section 1 into section 2. Dr. Swenberg also perceived the same tumor extending into section 3 (another new tissue section prepared for the re-examination), with Dr. Bolte agreeing. The latter individual had not reported the finding in his original diagnosis of section 3. Dr. Swenberg concurred with Dr. Bolte's diagnosis in section 1.

In view of the above findings, Dr. Dementi sought clarification via an April 14, 1999 phone conversation with the registrant's representative, Jellinek, Schwartz and Connolly, Inc. The question posed was: what explains the Study Pathologist's change of diagnosis from the original carcinoma of the olfactory epithelium in section 2, to a diagnosis which describes no tumor finding in section 2, to that of adenoma of the respiratory epithelium of another section. The response to that question as we have received it in the April 19 letter of Dr. Bolte (MRID 44837001) (copy attached) reads in part as follows: "When only sections from Levels 2 and 4 were available, a fragment of a neoplasm obviously originating from the respiratory epithelium, was seen in the nasal lumen. However, when additional sections were cut, this neoplasm was seen to be attached to the respiratory mucosa on level 1 and was identified to be an adenoma of respiratory epithelium." Further along, Dr. Bolte says: "At the time of the original reading (prior to that for the peer review) this neoplasm was categorized as a carcinoma of the *respiratory epithelium* (emphasis added), however, when the site of attachment was found, the neoplasm could be more appropriately evaluated and the diagnosis was changed to adenoma of respiratory epithelium." In this latter statement, Dr. Bolte's characterization of the tumor in question as being of the respiratory epithelium in the original reading is not consistent with the origional reading as reported in the origional study submission (MRID 43942901) (p. 4100).

It remains a curiosity in the mind of this reviewer as to why the tumor was originally identified as a carcinoma, and why of the olfactory epithelium.

Another question posed in the April 14 phone call concerned the diagnosis of the neoplasm in female rat #5503, from the high dose group. In the original study submission, the neoplasm was diagnosed as "squamous cell carcinoma arising from the squamous epithelium lining the alveolus of a tooth" (p. 5062) (copy appended), yet in the report submitted to Dr. Swenberg, the diagnosis for the same nasal tissue section read "palate: squamous cell carcinoma"(p. B-2237) (copy appended). In responding to the April 14 question, in his April 19 letter, Dr. Bolte says: "During my original evaluation of level 4 of the nasoturbinal tissues from the animal, I concluded that the squamous cell carcinoma may have originated from a tooth alveolus. During the peer review with Dr. Swenberg, we discussed the origin of this neoplasm and jointly concluded that the site of origin was the palate adjacent to the tooth." This response is clear except that on reading the report of the re-analysis written by Dr. Swenberg, one is led to the conclusion that the diagnosis of squamous cell carcinoma of the palate was the diagnosis submitted by the Study Pathologist to the Reviewing Pathologist prior to the latter's examining the slides. In other words, the diagnosis appears to have been revised prior to any discussions between the two pathologists, if this reviewer understands the submission correctly.

In view of uncertainties concerning the changes of diagnoses as discussed, additional more detailed descriptions of procedures followed in the re-examination were requested in the April 14 phone conversation. Specifically, were new slides for sections 2 and 4 for male rat #5040 possibly obtained along with sections 1, 3 and 5, that might explain differing diagnoses from the very original? Dr. Bolte has answered this question in his April 19 letter, saying the original slides from sections 2 and 4 were retained and not re-cut.

Upon further consideration of the results of this nasal tissue histopathology re-evaluation and peer review, and in consideration of the oral cavity tumors that were identified in the nasal tissue re-evaluation, additional clarification was sought from the registrant concerning the extent of the pathology evaluation of oral cavity tissues. Accordingly, in response to a July 19 request from Dr. Dementi, Ms Patricia Moe, product manager, requested the additional information from the registrant by phone conversation with Mr. Paul Whatling on July 21. A response provided by Dr. Bolte dated August 11 (copy appended)(MRID 44970600), was received officially under the September 30 cover letter of Mr. Blane Dahl to Ms Moe. In his response, Dr. Bolte indicated that all cavities, oral cavity included, received postmortem examinations for macroscopic abnormalities, and that the tissues associated with the oral cavity included the lips, gingiva, teeth, buccal mucosa, tongue and hard palate.

Dr. Bolte also provided additional historical control data for oral cavity neoplasms. In reference to the data base from the performing laboratory, Huntington Life Sciences, Dr. Bolte says: "Inhalation studies were selected since oral tissues are also examined with the nasoturbinal tissues. Four recent studies had 453 unexposed, control rats (227 males, 226 females). A squamous cell carcinoma arising from a tooth alveolus occurred in 1/227 males and a fibrosarcoma arising from peridontal tissue occurred in two rats, 1/227 males and 1/226 females. The rare occurrence of squamous cell carcinomas and fibrosarcomas are usually sequellae to peridontal disease. Peridontal disease, uncommon in aging and aged rats, is characterized by the presence of impacted food particles, inflammation ranging from acute to chronic, fibrosis and hyperplasia of the squamous epithelium lining the tooth alveolus. The severity of peridontal disease is variable and on occasion it can be severe with extensive squamous cell hyperplasia." We should note at this point that peridontal disease was not uncommon in the malathion combined chronic toxicity/carcinogenicity study, but occurred in all groups. Dr. Bolte also provides historical data for CD and Fischer 344 rats. Squamous cell tumors of the oral cavity are demonstrated to be very rare. Accordingly, the following incidences were provided. For CD rats: "Squamous cell carcinoma-hard palate: 1/1686 males. Squamous cell carcinoma-site not specified: 1/1686 males and 3/1691 females. Papilloma: lip: 1/1691 females."

For Fischer F344 rats (citing the NTP data base, 1990). "UNTREATED RATS: Squamous cell papilloma-site not specified: 1/1936 males, 1/1983 females. Squamous cell carcinoma-site not specified: 1/1936 males, 1/1983 females." "CORN OIL GAVAGE: Squamous cell papilloma-site not specified: 6/1949 males, 6/1950 females. Squamous cell carcinoma-site not specified: 0/1949 males, 0/1950 females."

In response to this submission, and at the request for still further clarification from this reviewer, Patricia Moe forwarded additional questions to the registrant in her letter of September 22, 1999, to

which Dr. Bolte responded via his letter of September 28 (copy appended)(MRID 44970501) submitted to the Agency under the September 30 cover letter of Blane Dahl.

Specifically, Dr. Bolte provided assurances that oral cavity tissues in question were examined macroscopically, but he advised that the oral cavity is not a protocol tissue. "Non-protocol tissues were listed only if macroscopic findings were noted, even if only one animal. Numerous tissues, including those associated with the oral cavity, not protocol required, were examined per Standard Operating Procedure (SOP); if there were no macroscopic findings, these tissues were not included in the incidence summary of macroscopic findings." We therefore conclude there were no *macroscopic* findings of the various tissues that constitute the oral cavity.

Dr. Bolte also affirmed that the nasal adenoma of male rat #5040, a neoplasma identified in the peer review as seen in nasal/turbinate sections 1,2 and 3, was not seen macroscopically. "Based on microscopic examination, the adenoma in the nasoturbinal tissues was very small. Due to its small size, it was unlikely that it would have been seen when examined macroscopically at the time that the nasoturbinal tissues were trimmed for processing toward microscopic evaluation." We understand from this that macroscopic examination of the nasal cavity occurs not before but during preparation of tissues for microscopic examination, and that this procedure may somewhat compromise macroscopic detection. Also, though the adenoma in question spanned three nasoturbinal sections, we acknowledge the possibility of its being of slender geometry which could explain its small size. Concerning the squamous cell carcinoma in the Group 2 female #2542, Dr. Bolte affirms it was not seen macroscopically. He explains this as due to an endophylic rather than an exophylic growth patter, which would make it difficult to be seen macroscopically during necropsy. While Dr. Swenberg characterized the tumor as being large, Dr. Bolte here says it would need to be massive in size to be seen macroscopically in the routine procedure.

Concerning clarification of the historical control data for the CD rat as presented in his August 11 correspondence, Dr. Bolte confirms incidences of squamous cell carcinoma of the hard palate among females was 0/1691 and the incidence of papilloma of the lip among males was 0/1686.

MEMORANDUM:

To: Auletta, Carol

From: Bolte, Henry /s/

Date: April 19, 1999

Re: 90-3641A: A 24-Month Oral Toxicity/Oncogenicity Study of Malathion in the Rat via Dietary Administration: Response to a telefax from Meena Sonawane to Carol Auletta, April 14, 1999.

Why Dr. Bolte changed his diagnosis from the first study to the recent peer reviewed submission:

> The request from the sponsor, directed by the EPA, to cut additional sections of nasoturbinal tissues from all rats on test resulted in an evaluation of the new sections and a re-evaluation of sections previously examined for the sake of consistency. As a result, some terminology, based on current knowledge, and a few findings in the original report were changed prior to submitting the results and report to Dr. Swenberg for peer review.

Were all sections recut?

> Additional sections requested by the sponsor were cut for all animals on test. Sections which had been previously cut were NOT re-cut. However, as stated above, when the new sections of nasoturbinal tissues were evaluated, all of the previous sections of nasoturbinal tissues were re-evaluated for the sake of consistency. Sections from levels 2 and 4 of the nasoturbinal tissues were the same as those evaluated for the original report; No new sections from levels 2 and 4 were recut (the only exceptions would have been a recut of sections not originally considered to be suitable for evaluation).

Animal #5040, Group V, (high dose) male:

> This animal still has a neoplasm involving the nasoturbinal tissues. When only sections from Levels 2 and 4 were available, a fragment of a neoplasm obviously originating from the respiratory epithelium, was seen in the nasal lumen.However, when additional sections were cut, this neoplasm was seen to be attached to the respiratory mucosa in level I and was identified to be an adenoma of respiratory epithelium. Dr Swenberg agreed with this at the time of the peer review and per the agreement of Drs Swenberg and Bolte, the fragments of this neoplasm in the lumen of levels 2 and 3 were described to be present in the nasal lumen. At the time of the original reading (prior to that for the peer review) this neoplasm was categorized as a carcinoma of respiratory epithelium, however, when the site of attachment was found, the neoplasm could be more appropriately evaluated and the diagnosis was changed to adenoma of respiratory epithelium.

Animal #5503 Group V (high dose) female:

> During my original evaluation of level 4 of the nasoturbinal tissues from the animal, I concluded that a squamous cell carcinoma may have originated from a tooth alveolus. During the peer review with Dr. Swenberg, we discussed the origin of this neoplasm and jointly concluded that the site of origin was the palate adjacent to the tooth.

Huntingdon
Life Sciences

MEMORANDUM:

TO: AULETTA, CAROL S. (STUDY DIRECTOP,
FROM: BOLTE, HENRY F. (STUDY PATHOLOGIST) /s/
CC: GOSSELIN, SYLVIE J.(VICE PRESIDENT OF RESEARCH)
DATE: 11, AUGUST, 1999
RE: 90-3641 -A 24-MONTH ORAL TOXICITY/ONCOGENICITY STUDY OF MALATHION IN THE RAT VIA DIETARY ADMINISTRTION

After euthanasia of test animals for postmortem examination, all external surfaces, all cavities, including the oral cavity, the eyes and all extremities are examined for the presence of macroscopic abnormalities. The tissues associated with the oral cavity include the lips, gingiva, teeth, buccal mucosa, tongue and hard palate. Macroscopic findings are noted and sampled for microscopic examination. For studies which require the microscopic examination of nasoturbinal tissues, the portion of the skull containing the nasoturbinal tissue, and attached soft tissue (paranasal and portions of the buccal mucosa) and the teeth, gingiva and hard palate are again examined macroscopically at the time that the tissues are trimmed; macroscopic findings are noted and processed for microscopic examination.

Sections of the skull containing the nasoturbinal tissues also contain the following: bone, bone marrow, the olfactory bulb of the brain, hard palate, teeth, gingiva, nasolacrimal ducts, paranasal soft tissue, buccal mucosa and on occasion skin. In inhalation studies, all are examined microscopically; this group of tissues are rarely examined in non-inhalation studies. For an occasional chronic study, the tongue is a protocol required tissue which is examined microscopically.

Neoplasms involving the tissues lining the oral cavity or arising from the tooth alveolus are uncommon. In inhalation studies, where the nasoturbinal and associated tissues are examined, they occasionally occur as a solitary incidental finding. In chronic studies involving a large number of animals exposed to escalating dose levels of a test article a finding, even if rare, with an incidence of one should not be considered to be treatment related regardless of the dose level at which it occurred. Most findings, inorder to be considered treatment related, usually have incidences greater than one and with some semblance of a dose related pattern.

The historical control data base at Huntington Life Sciences/Princeton was reviewed for the presence of neoplasms of tissues associated with the oral cavity. Inhalation studies were selected since oral tissues are also examined with the nasoturbinal tissues. Four recent studies had 453 unexposed, control rats (227 males, 226 females). A squamous cell carcinoma arising from a tooth alveolus occurred in 1/227 males and a fibrosarcoma arising from periodontal tissue occurred in two rats, 1/227 males and 1/226 females. The rare occurrence of squamous cell carcinomas and fibrosarcomas are usually sequellae to periodontal disease. Periodontal disease, uncommon in aging and aged rats, is characterized by the presence of impacted food particles, inflammation ranging from acute to chronic, fibrosis and hyperplasia of the squamous epithelium lining the tooth alveolus. The severity of periodontal disease is variable and on occasion it can be severe with extensive squamous cell hyperplasia.

Incidence of neoplasms of tissues associated with the oral cavity in untreated control rats have been reported for both the CD® and the Fisher 344 Rat[1,2] In the CD® rat, the incidence oral neoplasms in 3377 rats (1680 males, 1691 females) used in 26 long term studies initiated in December 1989 to April 1995 was as follows:

Squamous cell carcinoma-hard palate: 1/1686 males.
Squamous cell carcinoma-site not specified: 1/1686 males and 3/1691 females.
Papilloma: lip: 1/1691 females.

In the Fisher 344 rat, the incidence of oral neoplasm in 3919 untreated rats (1936 males, 1983 females) and in 3899 corn oil gavage treated rats (1949 males, 1950 females) was as follows:

UNTREATED RATS:

Squamous cell papilloma-site not specified: 1/1936 males, 1/1983 females.
Squamous cell carcinoma-site not specified: 1/1936 males, 1/1983 females.
Tooth-odontoma: 2/1936 males, 0/1983 females.

CORN OIL GAVAGE:

Squamous cell papilloma-site not specified: 6/1949 males, 6/1950 females.
Squamous cell carcinoma-site not specified: 0/1949 males, 0/1950 females.

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[1] Giknis, Mary L. A. and Clifford, Charles B.: Spontaneous neoplastic Lesions and Survival in Crl:CD® (SD)BR Rats Maintained on Dietary Restriction; Charles River Laboratories, 1998.
[2] Boorman, Gary A. *et al.::* Tumor Incidences in Fischer 344 Rats: NTP Historical Data; Pathology of the Fischer Rat; pp-555-564; Academic Press, Inc., New York, New York, 1990.

MEMORANDUM:

TO: AULETTA, CAROL S. (STUDY DIRECTOR)

FROM: BOLTE, HENRY F. (STUDY PATHOLOGIST) /S/

CC: GOSSELIN, SYLVIE J. (VICE PRESIDENT OF RESEARCH)

DATE: 28 September, 1999

RE: 90-3641; A 24-MONTH ORAL TOXICITY/ONCOGENICITY STUDY OF MALATHION IN THE RAT VIA DIETARY ADMINISTRTION: RESPONSE TO QUESTIONS SUBMITTED TO HENRY BOLTE, 23, SEPTEMBER, 1999.

1): Affirmation that the oral cavities (including tongue, mucosa, palate, gingiva, etc.) of all animals were examined macroscopically, and found to be negative consistent with laboratory records.

All tissue required per protocol were listed in the incidence summary of the macroscopic findings even if no macroscopic findings were noted. Non-protocol tissues were listed only if macroscopic findings were noted, even if only for one animal. Numerous tissues, including those associated with the oral cavity, not protocol required, were examined per Standard Operating Procedure (SOP); if there were no macroscopic findings, these tissues were not included in the incidence summary of macroscopic findings.

2a): Assurance that the adenoma seen in the tissues of the nasal cavity was not seen macroscopically.

Based on microscopic examination, the adenoma in the nasoturbinal tissues was very small. Due to its small size, it was unlikely that it would have been seen when examined macroscopically at the time that the nasoturbinal tissues were trimmed for processing toward microscopic evaluation.

2b): Assurance that the squamous cell carcinoma of the tooth alveolus was not seen macroscopically.

Squamous cell carcinomas arising from the tooth alveolus usually have an endophytic rather the exophytic growth pattern. Unless massive in size with considerable tissue disruption, which was not the case in this study, it is not likely to be seen macroscopically at the time of necropsy or at the time that the tissues are trimmed for processing toward microscopic evaluation.

3): Incidence of squamous cell carcinoma of the hard palate in females and papilloma of the lip in males:

The assumption that the incidence of squamous cell carcinoma of the hard palate among females was 0/1691 and that the incidence of papilloma of the lip among males was 0/1686 is correct.

Pages 10-13 of this attachment have been claimed confidential. They are releasable to persons who submit a signed “Affirmation of Non-Multinational Status” form.

ATTACHMENT 20

From: Brian Dementi 1/12/2000 8:32 AM
To: William Burnam/DC/USEPA/US@EPA
cc:
Subject: Re: Malathion memo of December 7, 1999

This is in response to your December 9 suggestion for an executive summary for use by the Cancer Assessment Review Committee (CARC) of the November 18, 1999 review on dialogue with Dr. Henry Bolte, study pathologist.

The CARC is reminded that in the origional study submission of the malathion combined chronic toxicity/carcinogenicity study in the F344 rat (MRID 43942901), nose/turbinate sections 2 and 4 were examined histopathologically. In the subsequent re-examination/peer review required by CARC, nose/turminate sections 1, 3 and 5 were obtained, and all five sections were then examined by the study pathologist, Dr. Henry Bolte, prior to the peer review involving Dr. James Swenberg (MRID 44782301).

In consideration of certain aspects of the pathology findings for tissues of the oral and nasal cavities as recorded in both the origional submission and the nasal tissue histopathology re-examination and peer review, follow-up questions with the study pathologist were deemed necessary. The questions and answers are paraphrased/summarized as follows.

1) What explains the differences between the study pathologist's (Dr. Bolte) diagnosis of nose/turbinate section 2 (high dose group male rat # 5040) variously reported as: a) *carcinoma* of the *olfactory epithelium* in the origional study submission; b) *hyperplasia, multi-focal, slight* of the *respiratory epithelium* in the nasal tissue re-examination as submitted to Dr. James Swenberg; and c) *adenoma* of the *respiratory epithelium*, originating in nose/turbinate section 1, after his meeting with Dr. Swenberg? Implicit in this question is what actually appears in section 2, and what would explain such a dramatic change in diagnosis?

By way of response, it appears that both pathologists agree there is an adenoma of the respiratory epithelium arising in section one (not available in the origional study submission) and extending into the lumen of sections two and three.

No explanation has been provided by Dr. Bolte for the remarkable differences in his origional diagnosis of carcinoma of the olfactory epithelium as contrasted with his subsequent diagnosis of *hyperplasia, multi-focal, slight* of the respiratory epithelium. The situtation is rendered more puzzling when in his letter of April 19, 1999, Dr. Bolte says: “When only sections from Levels 2 and 4 were available, a fragment of a neoplasm *obviously* (emphasis added) originating from the *respiratory* (emphasis added) epithelium, was seen in the nasal lumen. However, when additional sections were cut, this neoplasm was seen to be attached to the respiratory mucosa in level 1 and was identified to be an adenoma of respiratory epithelium.” Further along he says: “At the time of the origional reading (prior to that for the peer review) this neoplasm was categorized as a carcinoma of the *respiratory* (emphasis added) epithelium, however, when the site of attachment was found, the neoplasm could be more appropriately evaluated and the diagnosis was changed to adenoma of respiratory epithelium.” Hence, there remains an inexplicit dual change in diagnosis, from carcinoma to hyperplasia, and from olfactory epithelium to respiratory epithelium, in section 2 between Dr. Bolte’s origional and second diagnoses.

The CARC will recall that in the origional study submission (MRID43942901) a Dr. William Wooding was named as study pathologist and Dr. Bolte as Associate Director of Pathology, but in response to HED’s inquiry, we were advised that Dr. Wooding left the company after completing the interim pathology, and

Dr. Bolte performed the pathology at term. A revised personnel sheet for the study was submitted by the registrant's representative, reflection this change of duty. So we accept that Dr. Bolte performed all of the histopathology here being discussed.

This toxicologist defers to the CARC the question of any need for further clarification, but he would desire for the sake of the record a more complete explanation, or examination of the slide by yet another pathologist.

2) Why the change of tissue site location of the squamous cell carcinoma of the high dose group female rat # 5503, from that of the squamous epithelium lining the alveolus of a tooth to that of the palate? Dr. Bolte has answered that on consultation with Dr. Swenberg, the two pathologists agreed the tumor was of the palate adjacent to a tooth. Equivocation over the tumor's location is not unreasonable given that its location was at the interface of the tissue sites in question. Yet, it
seems from the temporal sequence of events that Dr. Bolte changed the diagnosis before submitting the data to Dr. Swenberg, at least insofar as this reviewer interprets the submission.

3) In view of the *seemingly* large size of the nasal adenoma (spanning three nasal sections) in male rat # 5040 and, the large size, according to Dr. Swenberg, of the squamous cell carcinoma of the squamous epithelium lining the alveolus of a tooth in the low dose group female rat # 2542, were these seen *macro*scopically? Dr. Bolte has responded that they were not seen macroscopically, saying the nasal adenoma was small, and the oral carcinoma had an endophytic growth pattern.

4) Were oral cavity tissues examined macroscopically? Dr. Bolte responded in the affirmative. He says that all cavities, oral cavity included, received postmortem examinations for macroscopic abnormalities, and that the tissues associated with the oral cavity included the lips, gingiva, teeth, buccal mucosa, tongue and hard palate. However, he indicated that non-protocol tissues, which includes the oral cavity, were listed in the incidence summary of macroscopic findings only if findings were noted. In the absence of any such listing, which prompted this question, we assume there were no macroscopic findings involving oral cavity tissues.

5) As to the questions concerning historical control data for squamous cell tumors of the oral cavity, Dr. Bolte cites the NTP data for the F344 rat with which CARC is already familiar. He also cites historical data for CD rats, where but one squamous cell carcinoma of the *palate* in 1686 males and none among 1691 females were reported. In addition, he reports a squamous cell carcinoma arising from a tooth alveolus in one male rat in a small historical control data base (227 males), presumably F344 rats, of the performing laboratory, Huntington Life Sciences. There were none in a similar number of females (226). Dr. Bolte says: "Inhalation studies were selected (for the historical data) since oral tissues are also examined with the nasoturbinal tissues." *This statement serves to underscore that oral tissues are not examined histopathologically with nasoturbinal tissues at this laboratory in the case of oral feeding studies.* The fact remains, as I have emphasized to the CARC previously (re: letters of B. Dementi to W. Burnam, July 13 and July 22, 1999) historical control data indicate that squamous cell tumors of the palate are extremely rare, at least to the extent oral tissues have been evaluated, and should not be discounted by the CARC, relative to nasal tumors, on the grounds of rarity.

Arguably, the four oral squamous cell tumors in the malathion study are of added concern with respect to the nasal tumors, as the former were identified *incidentally* in the nasal tissue histopathology assessment, which was not accompanied by full assessment of the oral cavity. *The findings of rare squamous cell tumors of the palate should indicate a full assessment of the oral cavity, and historical control data for oral cavity tissues not examined in this study should not be used to discount squamous cell tumors identified in the few oral tissues that were examined.* An additional curiosity resides with yet another statement of Dr.

Bolte in his August 11, 1999 letter. Namely:"Sections of the skull containing the nasoturbinal tissues also contain the following: bone, bone marrow, the olfactory bulb of the brain, hard palate, teeth, gingiva, nasolacrimal ducts, paranasal soft tissue, buccal mucosa and on occasion skin. In *inhalation* (emphasis added) studies, all are examined microscopically; this group of tissues are *rarely* (emphasis added) examined in non-inhalation studies." **Should the Agency interpret this to mean that these tissues, including the palate, gingiva and teeth, were not examined in the malathion study in a definitive way, and that consequently the squamous cell tumors that were identified, were simply particularly obvious?**

At the last two CARC meetings (September 30 and October 4, 1999) we discussed briefly whether the tongue and pharynx, as oral cavity tissues, were examined in the origional malathion study. We concluded the tongue had not been examined histopathologically, but had been mistakenly checked in the DER as having been examined. Also, I said at the last meeting the pharynx had been examined in the origional submission. On further scrutiny, I find that the origional study submission (MRID 43942901) does present data in the histopathology summary tables for the “pharynx” (e.g., p. 2943), though this tissue is not included in the protocol list of tissues to be examined (p. 46). Yet, the text discusses this particular data under the term nasopharynx (e.g., pp. 7, 84, 89).

The pharynx is common to both nasal (nasopharynx)and oral (oropharynx) passages. The nasopharynx (respiratory epithelium) (p. 89 of the study report) was examined along with nasalturbinates in the origional study. No additional nasopharyngeal tissue sections were cut in the nasal tissue re-examination. The oropharynx (squamous cell epithelium), which is the appropriate pharyngeal tissue to be examined in an assessment of oral cavity tissue responses, has not been evaluated histopathologically in this malathion study, as this reviewer understands the subject. So the fact remains, as explained in my letters to you of July 13 and July 22, among oral cavity tissues, only those of the palate and alveoli of teeth (perhaps including gingiva) were examined, incidentally, if you will, in what in fact was a nasal tissue evaluation. I would encourage the CARC to consider closely my understanding of the nature of the assment, as represented here, concerning the character of the histopathologic assessments of oral and nasal cavity tissues.

In the opinion of this toxicologist, affirmation is needed that oral tissues capable of being examined microscopically in the naso/turbinate sections, were in fact examined for *all* animals, and that naso/turbinate sections are indeed the sections that would be employed in an oral tissue histopathology assessment to identify possible tumorigenic responses of such tissues as palate, gingiva and tooth alveolus. By this I mean, are we confident we have reliable assessments for these particular tissue components of the oral cavity, not withstanding the fact that we lack assessments of other oral cavity tissues, such as those of the tongue, oropharynx and other oral mucosal tissues.

Again, squamous cell tumors of the palate and squamous epithelium lining the alveolus of a tooth appear to be essentially as rare as the nasal tumors, and could be viewed as of greater concern than the nasal cavity tumors since the former were identified in a nasal cavity assessment that provided only a partial assessment of the oral cavity. The fact that the macroscopic examination of the oral cavity was negative is encouraging, were it not for the fact that none of the squamous cell tumors was so identified. **Given the facts as presented, and taking into consideration the intimate contact of tissues of the oral cavity with the feed laced with the test material, this toxicologist would recommend for CARC's consideration, an audit of the oral cavity component of the study.**

ATTACHMENT 21

Jess Rowland
Executive Secretary
Cancer Assessment Review Committee

February 7, 2000

Please be advised I have read the February 2, 2000 Final Report of the Cancer Assessment Review Committee (CARC) on the Evaluation of the Carcinogenic Potential of Malathion. Since this final report incorporates as attachments the various memoranda and comments on earlier drafts of this report that I provided the committee, I have elected to sign the report, even though many differences remain, including some points of fact I noted in earlier drafts that have not been corrected.

The comments I provided on the October 28, 1999 draft *now* serve as my comments on the February 2 final report, as I see no evidence in reading the latter that the October 28 comments have been responded to in any way, this despite the fact that a) certain factual information remains incorrect, and b) inconsistencies remain.

As examples of what I refer to (and only a couple examples are here provided), please consider the following:

a) *In terms of the factual elements:* 1) the report says on p. vi, 3d paragraph: "The presence of one tumor per dose level at the lower doses of 50 ppm and 500 ppm was considered to be of biological significance .....", when in fact there were two tumors in both of those particular dose groups. Also, one of each of the two tumors was a carcinoma. Furthermore, in reference to the same paragraph of your report, I would consider as incorrect the claim that liver tumors were "mainly adenomas", when in fact across all dose groups the number of adenomas was eight and carcinomas five. Certainly the presence of malignant liver tumors should be acknowledged. I believe the paragraph as written would be, at best, misleading to persons who may receive or read only the Executive Summary. I should remind the committee that this endpoint is, in the committee's own assessment (p. viii), the principle endpoint driving the *likely human carcinogen* outcome, and, therefore, a peculiar burden rests with the committee to summarize it correctly in the Executive Summary; 2) A date of 1997 is given for the origional pathology report of the malathion carcinogenicity bioassay in the mouse (p. 4), while in my last two sets of comments on draft CARC reports, I noted the date for the study report was October 1994, and HED's review as February 1995.

b) *In terms of inconsistencies:* 1) Concerning thyroid follicular cell tumors in male rats, the September 20, 1999 CARC report conclusion (i.e. of the committee convened) was based on the fact that when the two excessive toxic doses are excluded, there is no increase in tumors. Yet, when I noted on the September 20 draft (p. 5), that the same argument supports as positive the finding of increased thyroid C-cell carcinoma in the lower dose groups, your subsequent report (October 28) *then* noted the argument in interpreting C-cell tumors, while invoking yet another reason to discount C-cell tumors at lower doses (namely, that adenoma versus carcinoma is difficult to differentiate, though I might add there has been no histopathology peer review to support the hypothesis in this very study, and no revisions have been made to tumor diagnoses of record). The October 28 draft also deleted the rationale in question in interpreting follicular cell tumors and employed instead other reasoning for discounting follicular cell tumors. The issue here is both the inconsistency of interpretation for the two tumor types, which is self-evident, and just how the members of the CARC interpreted the data. If the committee did employ the rationale given in your September 20 draft to discount the follicular cell tumorigenic response, then that rationale should stand in the CARC report as a matter of the historical record, subject to change only by re-consideration of the committee as a whole. Did the *committee* revise its rationale on follicular cell tumor interpretation at any subsequent meeting?; 2) In the October 28 draft, CARC concluded that the four nasal tumors are treatment related, but discounted the four squamous cell tumors of the oral cavity, apparently by 1)

treating the three of the palate as different from one of the squamous epithelium of the alveolus of a tooth, despite the fact these are combinable tumors, and 2) saying the three squamous cell tumors of the palate were not attributable to malathion due to lack of statistical significance and absence of a dose-response in either sex (p. 14). The inconsistency rests with the fact that the rare nasal tumors were not statistically significant nor dose-related, and yet, by contrast, in females, a squamous cell papilloma at 6000 ppm and a squamous cell carcinoma at 12000 ppm, constitutes some evidence of a dose-response in terms of progression. Furthermore, in the case of extremely rare tumors, a fact shared by both the nasal and oral cavity tumors, statistical significance is not required. Also, in the case of females, while one of the nasal tumors occurred at a dose not considered excessive, there were two such females with oral cavity squamous cell tumors at doses not considered excessive.

Again, as I have noted on earlier drafts, there are other errors and inconsistencies remaining beyond the two examples of each provided here that are recorded in various comments. These are a matter of the record, which hopefully interested individuals will appreciate.

I am compelled to note two additional observations pertaining to the final document not previously noted on my October 28 draft: 1) On p. 16, end of third paragraph, your report should note thyroid C-cell tumors were of concern in the earlier NCI studies as noted on p. 2 of your report; and 2) The report should mention in the first paragraph on p. 21, the leukemia concern in male rats in the 1996 malaoxon study (p. 24) and earlier NCI study of malathion in the F344 rat as noted (p. 2); the same concern rests with references to leukemia findings in other studies as mentioned in the 4th paragraph (p. 24) in discussing malaoxon.

Permit me to close by saying, though differences remain, I have enjoyed working with CARC members, and feel that I have many good friends on the committee.

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist/HED

The following attachments are not available electronically. Please see file copy.

ATTACHMENT 22

Jess Rowland February 9, 2000
Executive Secretary
Cancer Assessment Review Committee

Comments on February 2, 2000 CARC report on malathion.

In my memorandum to you dated October 28, 1999, I responded to a request from you for comments on the draft CARC report dated October 28, which I had received somewhat prior to that date. As I explained in my October 28 covering memorandum, in the interest of providing an expeditious response, I was appending the October 28 draft with my comments penned in the margins. Since that time I have received a January 13, 2000 draft circulated to the entire committee and following that, the February 2 final CARC report. Upon reading the latter two versions, I find the text to be essentially the same as that of the October 28 draft, an observation which you have confirmed. Therefore, the October 28 comments in effect become my comments directed to the February 2 report. For the sake of clarity and conciseness, I have elected to transcribe herein the comments penned in that October 28 draft report, to be reviewed by the committee and to be included among the other attachments to the final report. These comments track the February 2 report except for the frame shift in numbering of the five page Executive Summary from pp. viii-xii to pp. iv-viii in the final report. This is intended to replace my comments as submitted on your October 28 draft, but hopefully the October 28 covering memorandum to you will be retained.

My comments are rendered in italics (also bold for current embellishments), given by page in the October 28 draft CARC report:

viii - 3d paragraph: ....and marked brain (20 to 43%) cholinesterase inhibition in both sexes. *You should indicate brain cholinesterase inhibition was 20% @ 8000 ppm and 43% @ 16000 ppm for males, with similar responses in females, rather than lump these together, as I do not consider 20% to be "marked" or excessive enough to discount the 8000 ppm group, certainly. Other people might agree with me.*

ix - 1st paragraph: The Committee further concluded that there is evidence of carcinogenicity in female rats (but not males) which manifested as liver tumors at all dose levels and tumors of the nasal mucosa at 6000 ppm, although nasal tumors were also seen at 12000 ppm (a dose considered excessive). *Nasal tumors were also observed in males, one each, @ 6000 ppm and 12000 ppm.* ***In reference to my October 6 comments on your September 20, 1999 draft, you say ok for noting the male nasal tumor finding,*** *but didn't make the change.*

ix - 3rd paragraph: ....was slightly outside the historical control range and well above the mean value in a small historical control data base. *There is no large historical data base that is appropriate, since this was an 18-month study, while the NTP data base is for 2-year studies. The performing laboratory data base is very inadequate.*

x - 2nd paragraph: ....and marked brain (20 to 43%) cholinesterase inhibition. *As stated on p. viii, the 20-43% should not be represented for both 8000 and 16000 ppm, as 20% inhibition at 8000 ppm may not be viewed as excessive by many people. Your case is not as strong for 8000 ppm as for 16000 ppm, though I believe, as stated elsewhere, both are inadequate arguments to discount these doses.*

x - 4th paragraph: ....and that the liver tumor incidences at 6000 and 12000 ppm (although

considered to be excessive doses).... *Should be revised to say: (although 12000 ppm is considered to be an excessive dose)*

x - 6th paragraph: Thus, the relevant incidence for the tumor type in question is 2/4000 control males. *In the same NTP data base, the reported incidence for neoplasms of the nasoturbinal tissues was zero in approximatel 4000 control female F344 rats. (Jess, see p. 62 of the study DER for confirmation of this number)*

xi - 1st paragraph: However, the Committee concluded that a systemic effect could not unequivocally ruled out. *A local effect, as opposed to a systemic effect is less likely for the tumors in females which were identified in section 5, the most remote nasal region and where little other histopathology was seen. In my recall, the discussion was such that no evidence exists to conclude whether via inhalational or systemic route or both.*

xi - 3rd paragraph: ....because (1)....; (2)....; (3)....; (4)....; and/or (5).... *See discussion for each tumor type for rationale as not treatment-related. This lumping together of reasons confuses the reader as to reasons employed for each tumor type. The statements should be specific for each tumor, or referred to the text as I have suggested.*

xi - last paragraph: Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess carcinogenic potential. *Its fine to say CARC voted malaoxon was not carcinogenic, but you should acknowledge the positive trend, as well as the findings in the high dose group as being statistically significant for leukemia, and say why this finding was discounted: reason - say dosing was excessive, according to CARC.*

xii - 3rd paragraph: The Committee further observed that it is plausible that tumor occurrences in these studies are dose-limited (i.e., tumors are induced only at excessive doses), however, mode of action studies to demonstrate this hypoyhesis are not available. *This would not be consistent with the findings of rare liver tumors in females at both low dose levels**, without qualifying the reality of the biological significance of tumors in both lower dose groups.***

2 - 1st full paragraph: ....was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most *(actually three)* of the studies *(under review by NTP)*. *This statement needs to be revised to reflect NTP's conclusions. Thyroid C-cell tumors was a positive finding statistically (trend and high dose pairwise)(both sexes) in the malaoxon study.*

3 - 4th paragraph: There was also a significant.....for combined adenomas/carcinomas ($p<0.01$). *Though not statistically significant, the increase at 800 ppm was over 4-fold that of the control, and thus contributes to the remarkably positive trend ($p = 0.000$).*

3 - 5th paragraph: ....doses but not at the mid dose (800 ppm) *(though increased over 4-fold that of the control, 9% versus 2%; why do you not wish to acknowledge this simple truth?*

4 - Second line: As reported in the origional study report of 1997. *Study date: October 1994; date of DER: February 1995; **hence, 1997 is incorrect**)*

4 - Footnote e: Two males at 100 ppm had both an adenoma and a carcinoma*; a third and possibly a fourth male had two carcinomas of the liver; i.e. in this dose group there are three and possibly a fourth mouse exhibiting liver tumor multiplicity.*

5 - Footnote c: *Same revision need here as in footnote e on p. 4 given above.*

5 - last paragraph: Increased incidences of adenomas, carcinomas and combined adenomas/carcinomas were seen at 100 ppm and 800 ppm, but none of the increases showed either statistical significance or a dose-response relationship. *To the contrary, the 800 ppm group in my view evidences a clear dose trend with respect to the 8000 and 16000 ppm groups, i.e. control (7%), 800 ppm (16%), 8000 ppm (27%) and 16000 ppm (96%). The increase at 100 ppm (19 %) is anomalous and suggestive of a different mechanism, involving more carcinoma.*

6 - last paragraph: Dr. Brennenke, the consulting pathologist, commented that in the evaluation of carcinogenicity, "tumor bearing animal" counts as one *(statistically or numerically)* regardless of the number or multiplicity of any tumor type. *In comparing a control versus a dose group, it is well recognized that multiplicity is a weighing factor in tumorigenesis assessment, particularly in that multiplicity is evidence of earlier onset or increased rate of progression or development of the disease. Please see for example OSTP (1985). I do not understand why the committee does not wish to acknowledge the significance of multiplicity.* Although carcinomas were observed.....the incidences showed neither a dose-response relationship nor statistical significance at any dose level. *Carcinomas are rare (few) in the historical controls, yet many appear in this study.* In addition, tumor incidences at the two high doses should be considered carefully since these dose levels were determined to be excessive for assessing carcinogenicity..... *To be fair with your reasoning, to the extent you seek to downgrade the importance of findings at 8000 and 16000 ppm, you should acknowledge certain of the concerns that the findings at 100 ppm may be real, namely: 1) multiplicity; 2) carcinomas; 3) nearly positive pairwise (p = 0.075); 4) numerically increased incidence, nearly 3-fold; 5) possibly different mechanism at low dose; 6) female rat also exhibited liver tumor response, "cannot be discounted", in the same low dose range of 100/50 ppm, for hepatocellular adenoma and carcinoma; 7) profoundly positive dose trend in mice, p = 0.000. I stand amazed over the imbalance in your treatment in so positive a study.*

7 - 1st paragraph: In the 5 historical control studies, the incidences of liver cacinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in another study (6.4%). *In reference to my 10/6/99 comments on this point, you say "This is a comment". The point nonetheless is, what does the committee say, or what guidelines may there be that address the usefulness of a control data base, particularly when so small in number of studies and animals tested. Surely some comment here is merited. When a control data base is so weak, greater reliance must be placed on the contemporanous control.*

7 - 2nd paragraph: Also in the NCI study, among females, the combined adenomas/carcinomas incidences were *2% at 0 ppm,* 0% at 8000 ppm and 4% at 16000 ppm in contrast to the present study where the tumor incidences in females were *2% at 0 ppm,* 19% at 8000 ppm and 84% at 16000 ppm. The committee noted that the tumor responses in the present study at the same dose levels was more pronounced than those seen in the NCI study. *Suggested preferred sentence: The committee noted as inexplicable among females the absence of an hepatocellular tumorigenic response in the NCI study, versus the clear positive findings for females in the new study.*

9 - table 5 footnote: Incidences presented are the <u>total of the lesions</u> observed in the 5 sections of the nasal tissue. *I can see the advantage of totalling the findings from all 5 sections, however a disadvantage to this approach is that it does not reveal that section 5 had little pathology, yet it was in section 5 that both nasal tumors in females were found. This suggests the tumors were de novo and not secondary to other pathology, which is very important. You should acknowledge that little of these findings were in section 5.*

9 - last paragraph: At necropsy, liver "masses" were seen at all dose levels*, but not in the control.*

10 - 1st paragraph: The Committee further noted that the 8000 ppm.....and the 16,000 ppm.....dose was more than twice the Limit Dose. *You've still disregarded my note that these doses are not all that high, given the Guideline 5% of the diet cut off, now that the study is done.*

11 - 3rd paragraph: There were no statistically significant increases in hepatocellular tumors at any dose level in male rats. *Explain why males may be negative - dosing (mortality) too great for male assessment.*

11 - 4th paragraph: In addition, the incidences of these two tumor types *in the 100/50 and 500 ppm groups also* exceeded the historical control.....

11 - last paragraph: The Committee also concluded that the liver tumor incidences at 6000 ppm and at 12,000 ppm (although *12000 ppm was* considered to be *an* excessive doses *(dose))* provide positive evidence of carcinogenicity. *There was no NOEL for hepatocellular tumorigenicity in this study.*

12 - 1st paragraph: This conclusion was based on: 1)..... ***add*** *5) carcinomas also exceed; 6) extremely rare in females per NTP data base.*

12 - table 7: *As presented in the table, p values of* $\underline{<}$ *0.05 are in bold type. Values so close to p = 0.05, e.g. p = 0.063 and 0.085 as shown in the table merit some emphasis, such as grey highlight. An exceedingly rare tumor such as carcinoma of the liver of the female F344 rat need not be statistically significant at p = 0.05 to be real, but in this case the p values being very close to the 0.05 criterion should be considered real as supported statistically for such a rare tumor type.*

12 - last paragraph: This was a nasal tissue reevaluation, and oral tissue findings (tumors of the palate and *alveolus of the* tooth) were.....

13 - 1st paragraph: Therefore, the relevant incidence for the tumor type in question is 2/4000 control males. *As noted on your p. x, the NTP data base incidences of nasoturbinal tumors is zero in approximately 4000 control F344 female rats.*

14 - 3rd paragraph: The Committee postulated that direct contact with malathion (by volitilization from the feed *(no)* or by inhalation of the feed through the nose (*possibly yes))* was a plausible explanation for the nasal tumors; however, it was concluded that a systemic effect could not be unequivocally ruled out*., particularly since the nasal tumors in females were in the back of nose (section 5) where little other evidence of a local effect was observed. I prefer and believe more accurate is your paragraph in the September 20 draft (p. 11). I believe the committee was persuaded that malathion volitility is too low to be a significant factor here.* The Committee noted .....is required based on the *lack of NOAELs for cholinesterase inhibition and non-neoplastic lesions of nasal tissues in both the 2-week range-finding study (MRID 44554301) and the 90-day study (MRID 43266601) (HIARC report). The fact that nasal histopathology was observed after only 2-weeks of treatment would not have been identified in Guideline subchronic studies and is a matter of concern with respect to effects by inhalation.*

14 - 1st paragraph: Palate tumors were observed..... These tumors were not attributed to malathion treatment due to lack of statistical significance *(statistical significance is not required for rare tumors)*, and absence of a dose-response in either sex. *A papilloma at 6000 ppm and a*

*carcinoma at 12000 ppm is some evidence of a dose related response. There was no such trend for the nasal tumors**, and there were malignant tumors among those of the oral cavity**. You should remind the committee, the origional reason for discounting the oral tumors versus the nasal tumors was the alleged lack of rarity of the former. However, I explained in memoranda that the squamous cell tumors of the oral palate are as rare as nasal tumors in NTP's data base. So now other reasons, also incorrect, are cited. You should say that squamous cell tumors of the palate, like nasal tumors, are extremely rare in the NTP data base. Incidence appears to be zero in both sexes, or possibly but one such tumor exists in the entire NTP data base as explained in my memoranda to the chairman.*

14 - last paragraph: The Committee concluded that the thyroid follicular cell tumors are NOT treatment-related since there is neither a pair-wise significance nor a dose-response relationship for any tumor type.....; only a trend was seen for the combined tumors. *A trend is a dose-response relationship finding. Elevated mortality and competing toxicity at 6000 and 12000 ppm in males may have compromised <u>full</u> expression of these tumors at 6000 and 12000 ppm. Peak incidence arguably occurred somewhere between 500 and 6000 ppm, at a dose not tested. Jess, I find it very disturbing that in the earlier draft it was claimed that the conclusion was based on the fact that when the two excessive toxic doses (6000 and 12000 ppm) are excluded, there are no increases in tumors. Yet, when I noted that the same argument fails in the case of C-cell tumors, you delete the argument here. If the concept is important, as the committee has recognized it to be, you should adhere to it in both thyroid tumor cases. Also, did the full committee revise its vote?*

16 - 2nd paragraph: The Committee also observed that when the top two doses (6000 ppm and 12,000 ppm) were excluded (Table 10b) from the analysis, *as appropriate, particularly since these two dose groups have been discounted by the committee due to excessive dosing,* there was a dose-related increase (2%, 4% and 13% at 0 ppm, 50 ppm and 500 ppm, respectively), *yielding a remarkably positive trend, p = 0.006,* a pair-wise significance (p = 0.013) at 500 ppm, and the increases at both doses exceeded the mean historical control incidence (6/239; 2.5%) for carcinomas in male rats. However, the pathologist *(what pathologist, i.e. who said this and on what occasion? I recall Swenberg saying pituitary adenomas and carcinomas difficult, **but do not recall it being claimed by a pathologist at CARC meetings, and this is the first draft of CARC meetings in which this claim is recorded*)** stated that thyroid C-cell adenomas and carcinomas are difficult to differentiate. *The diagnoses have been rendered and not refuted by any peer review. Furthermore, there likely is a significant conversion of adenoma to carcinoma at 500 ppm, per discussion in my May 18, 1999 memorandum to Burnam.....*However, the Committee noted that although excessive mortality was also seen in females at the top dose (64% at 12,000 ppm) liver tumors were seen at this dose. *This is not to say incidence would not have been greater yet for liver had survival been longer, i.e. the finding was seen, but still may have been compromised. Also, consider leukemia (males) in this study for effects of 12000 ppm in mitigating expression.*

16 - 3rd paragraph: The combined tumors were determined to be the most appropriate tumor type for evaluation due to the difficulty in distinguishing the individual tumor types (i.e., adenomas and carcinomas). *I disagree with this assertion. Can you show me where in the record of any meeting this was voted on the basis of this principle, **furthermore diagnoses have not been revised by any independent examination of the slides by a pathologist.** Thyroid C-cell tumors were of principle concern as a finding in the earler NCI studies, particularly for the malaoxon F344 rat study. See your own list on p. 2 of this document. On this tumor type I stick with my comment on the earlier draft, p. 5 of my 10/6/99 comments. Also, you still do not acknowledge C-cell tumor multiplicity for the 500 ppm group in your reasoning. Furthermore, you say nothing about my May 18, 1999 memorandum to the chairman on C-cell tumors. In summary. Your comments on C-cell tumors are not balanced, presenting both sides of the question.*

20 - 1st paragraph: The Committee concluded that the testicular tumors are NOT treatment related. *Why did we perform the Peto test on this data if we were not prepared to honor the findings? The Peto test takes mortality into consideration. If conducted properly, as we assume it was in this case, the only explanation is that the tumor type, in mass (i.e. close to 100% incidence) in all groups was unexpected among animals of such high mortality. You do not need serial sectioning to perform the Peto test.* ***Furthermore, the burden of proof otherwise rests with those who denounce the findings for some hypothetical reason.***

21 - 1st paragraph: The Committee concluded that mononuclear cell tumors in male and female rats are NOT treatment related based on the lack of statistical significance at any dose level. *Please note and say that there was statistical significance for the increased female leukemia incidence at 100/50 ppm* ***(p = 0.025)****. Furthermore, while not quite significant by the p = 0.05 criterion, the increase at 500 ppm was significant at p = 0.059. This is very important in consideration of all of the data, males included, which I discussed in my April 27, 1999 memorandum to the chairman. Namely, that mortality due to leukemia among leukemia bearing animals was increased in a dosing-related manner, constituting evidence of increased progression under the OSTP (1985) definition of carcinogen. Also, leukemia was a finding at 2000 ppm in the recent malaoxon study and in the malathion NCI study (see p. 2 of your paper). All of these facts should be presented here to provide your audience a more balanced assessment.*

21 - 1st paragraph: .....and the 1996 malaoxon *(no!)* studies.

21 - 2nd topic: C. Non-Neoplastic Lesions. *The whole question of nasal tissue vulnerability to malathion is one which has not been adequately addressed by HIARC or CARC. While another inhalation study has been required that hopefully will shed more light on the subject, given our present knowledge, what can be said at this time concerning risk? It is uncertain whether the nasal histopathology in the rat (and mouse also) in the chronic feeding studies was a local effect or a systemic effect. I am inclined to believe both. In any case, the chronic feeding studies and the subchronic and dose range-finding inhalation studies all attest to a remarkable sensitivity of nasal tissues to malathion that has not been adequately addressed, particularly for inhalation exposures, the effect of which on nasal tissue would be exacerbated by oral ingestion. CARC has provided a Q* for nasal tumors in an oral feeding study, but does this address risk for persons exposed chronically by the inhalational route? This report should say something about this concern.*

21 - 3rd paragraph: Mortality was increased in males at 6000 ppm and in both sexes at 12000 ppm..... *The increase in mortality among males appears to extend down to 500 ppm, 47% mortality versus 33% in the control.*

24 - Table 20. Mononuclear Cell Leukemia in Rats Fed Malaoxon for 24 Months. *Add to table legend: Method of Peto, et al (1980) per September 22, 1997 letter of Huntingdon Life Sciences to Dr. Judy Hauswirth*

24 - 4th paragraph: The Committee concluded that mononuclear cell leukemia.....and F344 (1979, NCI-malaoxon) studies, *but was of concern in the NCI malathion F344 rat study (males) - see p. 2 of your report. Again, balance is important, both pro and con.*

25 - 1st paragraph: *Your numbers are now <u>good</u> and show a more remarkable effect at 2000 ppm than at 1000 ppm, particularly brain cholinesterase inhibition. It is not clear to me what criteria CARC follows in saying cholinesterase inhibition is excessive or not. Arguably, the blood enzyme*

inhibitions at 1000 ppm are excessive. For cancer assement, though, I really don't think so, absent clinical signs that are unacceptable.

28 - 2nd paragraph: In a subchronic inhalation study,.....was 2.01 mg/L (MRID 43266601). *HIARC is requiring another inhalation study to address absence of NOAELs and to further characterize nasal histopathologic responses.*

30 - 3rd paragraph: When compared with historical control ranges.....historical control range (0 to 6.4%). *You should show the mean as you did on p. 31, 2nd paragraph for the rat.* No carcinomas were seen at 16,000 ppm while the incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range, *and well above the mean value in a small historical data base of the performing laboratory. Unfortunately, NTP's data base is for full 2-year studies and cannot be used in this comparison.*

31 - 3rd paragraph: However, this conclusion was lessened since these tumors occurred in mice only at doses which caused severe plasma (90 to 95%) and red blood cell (92 to 96%) cholinesterase inhibition and marked brain (20 to 43%) cholinesterase inhibition. *Again, you should break out brain cholinesterase inhibition for 8000 and 16000 ppm. People may not be convinced that 20% inhibition at 8000 ppm is excessive such as to support discounting 8000 ppm as an excessive dose.*

31 - 4th paragraph: The occurrence of two tumors per dose level in the lower doses of 50 ppm and 500 ppm was considered to be of biological significance, *particularly in view of their rarity,* and increased the level of the Committee's concern.

32 - 2nd paragraph: Therefore, the relevant incidence for the tumor type (adenomas) in question is 2/4000 control males. *In the same NTP data base, the reported incidence for neoplasms of the nasoturbinal tissues was zero in approximately 4000 control female F344 rats.*

32 - 3rd paragraph: The Committee postulated that direct contact with malathion (by volatiliztion from the feed..... *See p. 13 for my comments on the volatilization issue. I question the committee accepted the idea.*

32 - last pragraph: C. Other Tumors *See my comments on p. xi in opposition to this language. You have glossed over the oral cavity squamous cell tumors, which are as rare as the nasal tumors. I remain concerned over the finding of eight exceedingly rare tumors in dosed groups only in the nasal tissue histopathology re-evaluation,* ***that did not include proper or complete histopathology assessment of the oral cavity.***

33 - 2nd paragraph: Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats *in the most recent (1996) study* ***(not owning a positive leukemia finding in males). Recall malaoxon was positive for C-cell tumors in the NCI F344 rat study, both sexes.***

33 - last paragraph: It should be noted that the classification of "likely human carcinogen" is based on..... doses not considered excessive. *It is somewhat inconsistent to then say:* it is plausible ..... tumors are induced only at excessive doses, **without qualifying the reality of the biological significance of tumors in both lower dose groups.**

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist/HED

ATTACHMENT 23

From: Brian Dementi April 10, 2000
To: William Burnam/DC/USEPA/US@EPA
cc: Marion Copley/DC/USEPA/US@EPA, John Carley/DC/USEPA/US@EPA, Dwight Welch/DC/USEPA/US@EPA, John Hirzy/DC/USEPA/US@EPA

Subject: Response to Dr. Copley's assessment of malathion CARC issues

Comments addressed to Attachment 1 of the March 31, 2000 CARC Package for the April 12 CARC meeting to consider Malathion

Attachment 1 of the indicated CARC package contains Dr. Marion Copley's partial responses to the issues raised by me as conveyed in late January and early February of this year to Mr. John Carley, OPP Director's staff. I was advised HED would review this material, but there has been considerable uncertainty as to when it should be expected, and just what opportunity I would be accorded for developing comment to that review. I had not expected the review this soon, and certainly believed I would have more opportunity to respond. I suspect that with Jellineck's recent submission of the Pathology Working Group (PWG) report on the rat liver tumor response, and the attendant high priority for CARC to consider this data, that a higher priority was assigned than previously to the review of my comments in order to include these at the CARC meeting. I suspect Dr. Copley has been under considerable pressure to review my comments, as evidenced by the facts that her review is not complete at this time and by my recent communications with her in our mutual participation in the expedited review of the PWG submission. I certainly would like to have had more time to respond to her review of my comments, particularly after her work has been completed. Her March 30 report says: "However, the response to reference 22, will be completed subsequent to the completion of this memorandum due to the large number of comments (about 50)." Actually. I would desire HED's responses to these comments before the CARC meeting, as they convey an enormous amount of information on the inconsistencies of reasoning and presentation of factual information in the CARC report. However, given the circumstances under which Dr. Copley and I must currently operate, I would like to offer for the CARC's consideration the following very condensed responses to Dr. Copley's views as presented in the form of her Attachment 1. I must reserve for the future, more extensive comment on her final report whenever that appears.

<u>Items as presented in Attachment 1</u>

1) Mouse liver tumors

*The combined responses,**at the high doses**, was driven by adenomas. The response in the lower, particularly the lowest, dose groups is driven by both tumor types. The highly statistically significant trend (p = 0.000) is undoubtedly contributed to by the combined tumor type effects at the lower dose levels, which in the case of the lowest dose group, approaches statistical significance (p = 0.075). There is a vast dose range in this study, 100 ppm to 16000 ppm. There is good reason to suspect, therefore, that different mechanisms of carcinogenicity may operate at the extreme ends of the dose range, which may yield, for whatever metabolic reason, essentially adenomas at the highest dose, but involve induction of carcinomas and/or progression of adenomas to carcinomas at lower levels. It is inconsistent with a conservative public health philosophy to disregard findings at the lowest dose given the positive trend, near pairwise positive comparison, presence of malignant tumors, multiplicity, and evidence in this group of a more advanced stage in the "natural history of neoplasia" for liver tumors as discussed in the background papers. Many of these particular concerns are not addressed in Attachment 1.*

The Committee will recall this mouse study received a PWG review, and that as of that review half of the carcinomas were downgraded to adenomas. Even after that purge, ***eight carcinomas*** *remain among the 100-8000 ppm dose groups, as opposed to none in the control and highest dose groups, while in the highest dose group the incidence of adenomas is 96% as opposed to 7% in the control. Clearly there is a peculiarity in the character of the dose response in this study. In the entire historical control data base for the performing laboratory there are but five studies, wherein a total of but 4 male mice with liver carcinomas is to be found, and these not having been subjected to the scrutiny of the PWG which downgraded eight carcinomas in the malathion study. Is there not anyone on the CARC who shares my concern that these historical carcinomas may not survive the strict scrutiny of the PWG that examined the malathion study? In any case, it is my opinion that it is inappropriate to cite this meager, non-PWG evaluated and somewhat old historical data base in concluding anything about the malathion study other than that carcinoma is a real concern in the malathion study. I believe Attachment 1 should have noted that we cannot refer to the NTP historical data base for assistance in the interpretation, because that data base is for the full 2-year mouse study, whereas the malathion study was an 18-month study. For these various reasons, the contemporaneous control must bear the burden of control (i.e. the historical control is essentially useless post-PWG of the malathion study) for this study.*

In reference to Dr. Copley's comparison between the mouse and rat study liver findings (p. 4), I would respond as follows: 1) I do not agree that carcinoma in the 18-month mouse study, particularly that which survived the mouse PWG, can be considered a common tumor type; 2) I do not agree the historical control data for the performing laboratory in the case of the mouse study, particularly after the PWG assessment, should be considered relevant; 3) agree, except with the statement that in the mouse study, control incidence was unusually low, given the problems with the historical data base.

As discussed elsewhere in my comments, I do not concur with discounting dose levels as excessive based on cholinesterase inhibition in asymptomatic (cholinergically) animals, and that to do so compromises testing at high doses called for in cancer bioassays, and thus precludes identifying potential human carcinogens. According to EPA's Cancer Risk Assessment Guidelines, these tumors should not be discounted unless it can be shown they were due to excessive toxicity as opposed to the carcinogenicity oif the test material.

Concerning the more remarkable effects among female mice in the more recent bioassay versus that of the earlier NCI study at the same dose level, 8000 and 16000 ppm, in the quoted paragraph it would be appropriate to show the control incidences for males and females, which across this span of years remained at about 2%. The profound differences in response among females at these doses should raise some concerns. For example, were any conditions of testing, or the nature of the test material used contributive? Was cholinesterase inhibition different in the two studies? The NCI study was in-life for 95 weeks (dosing for 80 weeks), while the recent study was for 78-weeks. In my opinion, it is disparate findings such as these which suggest findings in one study do not mitigate findings in another study; or once a study is positive, it can be discounted only by several negative studies.

2) Thyroid c-cell tumorigenic response in the male rat

The comments here do not address the issue raised in my letter of May 18, 1999 to William Burnam. Specifically, that the study mirrors the kind of response disuussed in McConnell, et al (1986), wherein there is evidence at the 500 ppm dose level of progression to carcinoma,

*though combined incidences of adenoma and carcinoma at this dose level were not increased. The issue is **progression** as evidence of carcinogenicity, and a positive finding is supported by a statistically significant increase in carcinoma at that dose. Although statistics were not performed on the relative incidences of progression, per se, I believe it self-evident that it would be similarly statistically significant. Again, this finding mirrors the kind of response McConnell et al was focusing on, and one that CARC should be leery of, i.e. how such progression could be easily missed in examining combined tumor data only. Yet, CARC has opted to rely on the combined data for its conclusion on this end point. I profoundly disagree with discounting the carcinoma findings on the basis of CARC's rationale that it is difficult to distinguish adenoma from carcinoma for this tumor type, pending an actual down-grading of this tumor type by inspection. We have as a matter of record these findings, and who knows how definitive the diagnoses may be. It is somewhat of an insult to the performing pathologist to proclaim, without confirmation, his diagnoses as non-definitive, where his findings may in fact be quite clear. As long as the carcinomas in question remain a matter of record, CARC's interpretation does not hold water.*

I have also indicated a concern over the use of less remarkable findings at the much higher doses of 6000 and 12000 ppm, which CARC concluded to be excessive doses, to rationalize away the findings at 500 ppm. In consideration of this conclusion, there is the argument that one should not accept as negative a study absent findings at excessive doses. The same should hold true for using less remarkable findings at excessive doses to discount findings at doses considered acceptable, which was the logic employed by CARC in this case. Finally, the CARC concluded as stated in Dr. Copley's review that at the 6000 ppm dose level, there were still 43 rats considered to be at risk. Actually, while 43 may have been at risk, they were not at risk for as long, given the high mortality in this group. However, that is really not the point. The point is that competing toxicity at 6000 ppm may have compromised expression of this response. The dose group has been declared excessive, and should not be used in a selective manner to support or refute this or that finding. This would constitute a no win or loose option for the use of this study, depending upon ones perspectives or desires. Alternatively, to the extent this study is not considered positive for c-cell tumorigenic response at 500 ppm, a dose level considered acceptable by the CARC, the study should be considered unacceptable for assessing c-cell tumorigenicity in males.

The bottom line is that a positive finding exists for this end point at 500 ppm that has not been successfully refuted, and is of concern because it finds expression in the next-to-the-low dose group, and if true, would be of enhanced concern from the public health perspective over findings at the high dose levels, where interpretation is more confounded by high dose anomalies, and less relevant in terms of human exposures.

3) Thyroid follicular cell tumors

Concerning the first sentence under "Response", a statistically significant trend establishes a dose-respons, contrary to what is said. In this study, there was a positive trend ($p = 0.035$) and a nearly positive ($p = 0.077$) three fold response versus the control at 6000 ppm. Be it agreed that to suggest competing toxicity may have dampened or compromised full expression is speculative. But this is no more egregious than speculating away c-cell carcinomas as discussed above. Nonetheless, CARC has declared the top two doses excessive, and therefore we might expect based upon the well recognized principles of competing toxicity that full tumorigenic responses may be compromised. There remains evidence even under these circumstances, of a dosing-related response, for which there is in effect a blackout in assessment in the wide dose range of 500 ppm to 6000 ppm, i.e. in a region where competing toxicity may exert less influence to compromise the response.

Admittedly, this is speculation, but not unreasonable. It is consistent with our duty, and cannot be refuted by data in the existing study, which again, for this reason the study may be unacceptable. It is axiomatic that if one should not accept as negative a study absent effects at excessive doses, he certainly should not accept as negative a study exhibiting marginal effects at excessive doses.

4) Leukemia in the rat: interpretation of evidence under OSTP (1985)'s definition of carcinogen

I would reference in particular my April 27, 1999 memorandum to William Burnam. In presenting an argument that progression in the case of leukemia was evident among male rats, I should note discussing this observation of a dosing related increased incidence of mortality due to leukemia among leukemia bearing animals with Dr. Robert Maronpot, at NTP, who very readily render the opinion the finding would be meaningful at NTP as evidence of carcinogenicity under the concept of compound induced progression. I should note that while Dr. Maronpot has not seen the data, I did consult with him, and therefore the interpretation offered by me is not without some concurrence of an outside expert.

I have no further comments at this time as this issue is moved along for consideration by CARC, except to say this endpoint perhaps more than any other illustrates the compromising effect of excessive toxicity, where at the 12000 ppm level, incidence was but one compared to many in other groups. While it may be true we do not know the mechanism explaining why the expression of this tumor type was, in effect, mitigated at 12000 ppm, nevertheless it should raise our concerns about evaluating this tumorigenic response at such doses, including 6000 ppm, and whether the study is acceptable for evaluating this response in males, and could be a reason to seriously consider the findings in females in the lower dose range. This was a tumor type of concern in the earlier NCI study in the F344 rat, and was a finding in the recent malaoxon study.

5) Interstitial cell testicular tumor in the rat

Under "Response", Dr. Copley says: "For this tumor type, the historical spontaneous occurrence approaches 100% by the end of a study." However, there is no available data to say that incidences approaching 100% occur earlier in the study, or better still to show the spontaneous influx of these tumors over the course of time. The key question is not whether these tumors naturally occur at earlier time points, but the point at which they become essentially universal findings. As I understand the Peto test, the results are statistically significant because more tumors than expected were seen earlier, i.e. tumors ***in abundance*** *were seen earlier in dosed groups. That argument aside as evidence of a positive response, the CARC has argued we do not have serial sacrifice data to establish decreased latency, i.e. we have no way of knowing the comparative rates at which this tumorigenic response occurrs(ed) in control relative to treated animals. What is being referred to is, in effect, mechanistic data, i.e. the absence of such data. Confronted with the positive findings as presented in the study report and essentially confirmed by HED's Peto test, simply stated the study is positive until mechanistic data is submitteed, which is the registrant's responsibility to provide, once the need for such data has been identified. On the other hand, it is inappropriate for CARC to note the absence of the needed data, and then conclude the findings are not real because the need data is absent. Again, it is the registrant's responsibility to provide mechanistic data that may explain away positive findings*

6) Rat nasal tissue histopathology and tumorigenic response in the rat

The revisions referred to under"Response" need to be seen by the Committee now, not after the April meeting, as they serve to illustrate flawed reasoning. The term "esthesioneural" has not been discussed at CARC meetings, as I recall. I would appreciate the opportunity to comment on this tumorigenic finding. While it might not be combinable with the other nasal tumors, this particular tumor type as I have discussed in supporting documentation, is utterly rare. As I recall there is not a single incident in NTP's data base or the performing labs data base. One was seen in a malathion treatment group in the NCI study conducted in the late seventies in the Osborne -Mendel rat.

The report says: "Based on the data currently in house, the CARC can not develop (as requested, I should actually say as observed, by Dr. Dementi) in interim ....." Further along Dr. Copley says: "Therefore.....I feel the CARC is taking the most conservative approach by considering there to be a potential carcinogenic risk by exposure from the inhalation route." I should add, as long as that classification stands. The Registration Standard (1988) for malathion required an inhalation study on the grounds there was sufficient exposure by this route. Another study is outstanding as required by HIARC, due to absence of NOELs for cholinesterase inhibition and nasal histopathology. Also in the HIARC report there remains unaddressed the question of the need for a carcinogenicity study by the inhalational route of exposure.

7) Oral cavity assessment for tumorigenic response

Under "Response", Dr. Copley says in the last sentence, p.18, "Therefore, I feel that it is difficult to attribute any biological significance to the occurrence of a single tumor occurring only at the low dose." I would agree with that statement were this not an extremely rare tumor for the palate, and were there not three such rare squamous cell tumors seen in females. The facts that they occurred in females (one in the low dose group there), and oral tissues have not been adequately evaluated histopathologically, combine to make this tumor much more suspect as treatment related.

Rare tumors need not occur in a treatment related manner to be of significance, as is true I feel of the one tumor of the olfactory epithelium mentioned previously, where there was extensive nasal histopathology and the other tumor types identified in the nasal (respiratory) epithelium, a target tissue.

In the first sentence on page 19, the view is expressed that historical control values presented by me are misleading, and that all squamous cell tumors of the oral cavity should be considered together. In response to this I must affirm not having been "misleading" to anyone, here or anywhere else in this mass of information. A more appropriate word, absent sufficient reason to justify the word misleading, would be the word "mistaken", if indeed that can be substantiated, which I do not think it can be. I agree that if histopathology were available in the case of the malathion study for the entire oral cavity, historical control data for the entire oral cavity would constitute the proper frame of reference. However, until we have such data, the relevant historical control data to consider is that component (oral mucosa of which palate is a constituent) of the overall oral cavity, which was examined incidentally in what, in fact, was a nasal tissue histopathology assessment in the malathion study. I regret this may not be clear in Ref. 20 cited by Dr. Copley, but I believe it is clear. If one examines NTP's historical data base, the incidences of tumors of the oral cavity derive from the sum of those for oral mucosa, tongue, pharynx, tooth and gingiva. The point I have endeavored to make is that until we have data in the malathion study on the ***entire*** *oral cavity, historical incidences observed for such tissues as the tongue, pharynx and other regions of the oral mucosa (beyond that of the palate), not examined in the malathion study,*

should not be used. The only legitimate frame of reference among oral cavity tissues to address rarity are those actually examined in the malathion study. Should this reasoning be incorrect, it should be characterized as mistaken, or better yet, an error of concept, not "misleading".

Further along, Dr. Copley says: "We do not know how many had slides with incidental negative oral tissue (I would be more specific and say the palate and alveoli of teeth) present since this was not reported. In fact, I would emphasize we cannot rely upon the nasal tissue histopathology to have properly characteried even the palate and alveoli, let alone the other oral cavity tissues we know were not examined microscopically, such as tongue, pharynx and wider regions of the oral mucosa.

Regarding Dr. Copley's last sentence on p. 19, in my view, ***all*** *tumorigenic responses should be properly evaluate, regardless of what impact they may be perceived to have on cancer classification. In this particular instance, proper evaluation may firm up a low dose effect, which could mitigate the argument that effects are limited to high doses even if "likely" remained the call. Conceivably, the Q* could change as well.*

8) Tumorigenicity (several end points) in the low dose groups...

I do not concur with Dr. Copley's overall appraisal of the importance of low dose effects. If the compound is carcinogenic at doses as low as 50 or 100 ppm, the concern for the public health, considering the doses are so low [food tolerances for malathion are 8 ppm (40 CFR 180.111)], is not addressed by Q assessments weighted heavily by findings at much higher doses. The low doses of 50 and 100 ppm in the malathion studies would, in my view, not ordinarily be tested along with doses as high as 12000 or 16000 ppm, were this not a cholinesterase inhibitor and a NOEL for inhibition was being sought. Also, as stated earlier one should question whether but one mechanism of carcinogenicity should be expected across such a vast dose range of 100/50 ppm to 12000-16000 ppm as would be the interpretation implicit in the Q* assessment as currently computed.*

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9) Decisions to discount dose levels as excessive for carcinogenicity assessment based on cholinesterase inhibition.

Under "<u>Response</u>", in the 3d paragraph, cholinesterase inhibition and mortality in the malaoxon study at 1000 ppm should be presented along with that for the 2000 ppm group, as I do not accept, in the absence of any guidelines for the use of cholinesterase data and mortality to determine whether dosing is excessive, close inspection of the data there is clear justification that malaoxon at 2000 ppm was excessive while not so at 1000 ppm.

In the 4th paragraph, brain cholinesterase inhibition in the mouse study at 8000 and 16000 ppm is presented as "20 to 43%". In my comments to the CARC report wherein these figures were given, I noted in response that in reference to the male data, the range should be broken out to show that inhibition was 20% (not statisically significant) for the 8000 ppm group and 43% (statistically significant) for the 16000 ppm group at term, and should not have been consolidated. I do not consider 20% inhibition as rising to a level sufficient to support cholinesterase inhibition as evidence of excessive toxicity, particularly in the absence of statistical significance and absence of cholinergic clinical sign, and where CARC places more emphasis on brain cholinesterase inhibition in making the judgement per Dr. Copley's 2nd paragraph. In other words, my view is that the 8000 ppm and 16000 ppm groups should not be consolidated as excessive doses, but that 8000 ppm should certainly not be considered excessive, and that CARC's justification for including both as excessive is

unsubstantiated.

In her last paragraph, p. 22, Dr. Copley quotes the Cancer Guidlines. The Guidelines also say that ***tumorigenic responses at excessive doses should not be discounted unless the responses have been shown to be due solely to excessive toxicity as opposed to tumorigenicity of the test material.*** *She also says: "Whether the response indicates an adequate dose or an excessive dose depends on the magnitude of the response." In this particular case of the mouse study, CARC is on shaky ground considering brain cholinesterase inhibition of 20% in the 8000 ppm group, in the absence of clinical signs, as of sufficient magnitude to support an argument of excessive dosing. The argument is more substantial based on the 43% inhibition at 16000 ppm, but I actually do not agree with that interpretation. In my view, animals have a remarkable capacity to down-regulate cholinergic reseptors in maintaining normalcy in the presence of substantial cholinesterase inhibition. To wit in this case where there are no cholinergic signs. They do just fine and serve well in a cancer bioassay. I do believe these animals are compromised in terms of cognitive performances, and would be subject to expressions of various responses following cholinergic pharmacologic challenges, but this is relative to dose and I don't believe CARC can defend itself in concluding dosing was excessive for the 8000 ppm dose group in the malathion study.*

Through the period of time we have been considering malathion, CARC has mellowed somewhat on the interpretation of the mouse study. As I perceived it, early in the process, cholinesterase inhibition was being cited to discount the high dose groups, and to say only doses of 800 ppm and below were acceptable. Later on, the CARC seemed to me to be assigning more weight to the findings at 8000-16000 ppm in the weight-of-the-evidence. It is still not entirely clear to me how much weight is being given these liver tumor findings, but the point I have made in more recent times, and at this point, is that the 8000 ppm and 16000 ppm groups should be treated differentially, with findings at 8000 ppm being more in the category of acceptable as positive for tumorigenicity. The study is problematic as the Agency required the 8000 and 16000 ppm doses to address findings at these same dose levels in the earlier NCI study. It is unfortunate a dose level somewhere between 800 and 8000 ppm was not tested.

10) Acceptability of OSTP's (1985) definition of carcinogen, and if considered acceptable, the rigor of its application in CARC's interpretation of the malathion studies.

The response appears to be one in support of the said definition, yet there is little in these comments illustrating the CARC's utilization of the principles of the second aspect of the definition. For example: a) In my discussion of the male mouse liver tumor response in the lowest dose group (100 ppm), the following have been noted in support of a positive finding relative to the control group: multiplicity, the presence of carcinomas, and large tumors as determined macroscopically, all of which point to a more advanced stage in the "natural history of neoplasia" in the low dose group. Yet, ignoring these facts CARC has rendered its interpretation on the absence of statistical significance in the low dose group despite the clear positive trend and numerical increase in tumors of the low dose group; b) In the case of c-cell tumorigenic response in the 500 ppm male rat group, multiplicity in addition to progression to neoplasia has been emphasized, with little recognition from the CARC as to its realization of the importance of these findings as added evidence of carcinogenicity. There are other examples.
Concerning the question of serial sacrifices, I do not consider these essential to establish decreased latency, when progression in a given group may be evident by significant

increased incidence of malignant tumors. Certainly, serial sacrifices would be most beneficial, and it is encumbent upon the registrant to provide such data should he hope to persuade that other evidence of progression already present is the data is somehow misconstrued as evidence of decreased latency or increased progression. One does not simply witness evidence of progression, and then disown honoring that evidence on the grounds that the best confirmatory evidence is not available, and leave it at that. In the interest of the public health and in view of the fact that the EPA should be conservative in its judgements, the Agency must accept the evidence of progression, until discounted by more substantial mechanistic data, which is the duty of the registrant to provide should he desire another interpretive outcome.

11) Incorporation of tumorigenic findings or the absence (or reduced incidences) of the same, at doses considered excessive.

I do not feel as though Dr. Copley's response addresses the concerns I have expressed over CARC's inconsistent and improper characterization (judgement rendered) pertaining to tumorigenic responses at excessive doses. The best expression of my views is to be found in Dr. Copley's reference 18 (my November 12, 1999 memorandum to William Burnam). Specifically, on the one hand, as contraindicated in EPA Cancer Assessment Guidelines, ***discounting*** *positive findings without demonstrating these were due solely to excessive toxicity as opposed to the tumorigenicity of the test material at doses considered excessive by CARC (e.g., liver tumors in the rat (female) and in the mouse (both sexes), and on the other hand* ***accepting*** *less remarkable tumorigenic responses at excessive doses in discounting a positive effect at the lower dose level (dose level considered acceptable by CARC, and perphaps harboring more importance in terms of the public health concerns because of the low dose involved)(e.g. thyroid c-cell tumor progression to carcinoma, accompanied by multiplicity in the male rat). The converse judgement should have been held to be true in both interpretations, according to the principles I have documented in the memoranda provided the CARC Chairman, which I trust the Committee will carefully consider.*

12) Application of general principles of competing toxicity and increased mortality in mitigating expression at excessive doses of a tumorigenic dose-response occurring at acceptable lower doses.

I have little further to say in addition to the views expressed in my memoranda to the committee Chairman, except that in reference to the Cancer Guidelines quote as cited, namely, "...(c) If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity." , I do not feel this principle, true though it may be, is applicable to the concerns being raised, where a) positive findings were in fact seen in certain instances at lower doses considered acceptable; b) where in other instances positive findings were observed at doses considered excessive by CARC; and c) where it is questionable whether to characterize as appropriate, the spacing of lower doses that were considered acceptable.

13) Acceptability of the rat combined chronic toxicity/carcinogenicity study to evaluate carcinogenic potential in male rats.

In the last sentence under "Response", Dr. Copley says: "I feel that the data would have supported the 500 ppm dose as adequate in the hypothetical situtation where it was the high dose in the study." I would agree with this to the extent the study is considered positive, but

not if concluded to be negative. If negative, the 500 ppm dose level, considered in contrast with an acceptable dose so high in females as 6000 ppm, and in view of the higher doses employed in the earlier NCI studies, cannot be considered acceptable. If males cannot be tested at doses higher than 500 ppm in the F344 rat due to increased mortality and competing toxicity, I would have to conclude this rat model to be unacceptable for the evaluation of malathion at sufficiently high doses as mandated by principles of carcinogenicity testing. To the extent the study is deemed negative, in order to achieve testing in male rats at a dose level somewhere within the range 500-6000 ppm, if doses higher than 500 ppm are not achievable with the F344 rat, another model should be employed before accepting this study as satisfying as a negative study in the male rat.

Dr. Copley also says: "....any additional information would not alter the cancer assessment and classification which is already, 'likely human carcinogen'". As I have indicated earlier in these comments, each study or tumor type should be pursued to completion. One cannot predict what impact proper testing may have on the interpretation of the carcinogenic potential. The Q is not final until testing is complete. Findings at lower doses may be identified, and so on.*

14) Adequacy of Q* method to address risks posed by low dose tumorigenic findings, e.g., liver tumors in the female rat at 100/50 ppm, in the absence of a NOEL.

In the interest of the public health, if malathion exhibits tumorigenic responses at low doses that appear more remarkable than would be expected from responses observed at higher doses, possibly suggesting a different mechanism from that at higher doses, it seems obvious to me one cannot treat risks properly with a single model which in effect acknowledges but one mechanism. This is more than a philosophical question, but one of real and proper risk assessment for malathion. I stick with my request for expert comment addressing my concerns.

ATTACHMENT 24

Dr. Marion Copley April 24, 2000
RAB #1
HED **[note added: Attachment 25 cited in this memo are actually Att. 23]**

Re: Comments to the Draft April 2000 *CANCER ASSESSMENT DOCUMENT #2:* Evaluation of the Carcinogenic Potential of Malathion

In all fairness, I would request that a qualifying statement concerning my involvement be introduced into the document at a point where my name first appears in the text, saying in effect: ***The Attachments by Dr. Dementi were drafted in consideration of the data base as it existed prior to evaluation of Cheminova's submission of the Pathology Working Group (PWG) Report on the female F344 rat liver tumor findings, the present comments, of course, excepted.***

Before proceeding with comments on this final CARC Report, be it known that in my experience as toxicologist on malathion there was not enough time allocated for preparation for the April 12 CARC meeting. The constraints of time continue to be manifested in the turn around time permitted for this CARC draft, having received it April 19, with a due date of April 21 (April 24 at 9 AM the latest). The haste with which the recently received Pathology Working Group (PWG) Report on female rat liver tumor response has been taken before the CARC (April 12), without a complete HED review of the report, should not be considered acceptable, and in my view, a vote should not have been taken on the cancer classification absent that review.

In order to facilitate an expedited response, my approach in rendering comment on this draft final CARC report will mainly be that of referring to previous comments that appear in various attachments to this CARC report. A principle set of comments on the February 2, 2000 draft CARC report are located as Attachment 22. When citing Attachment 22 in the present comments, I will be including, parenthetically, Attachments 8 and 16 as providing similar comments directed to earlier CARC draft reports. Another important set of comments appear under **Attachment 25 (not yet incorporated as an attachment to the draft CARC report as I am requesting, in order to facilitate developing the present response to the draft CARC Report)**, which is my April 10 response to Dr. Copley's March 30 assessment of my earlier conclusions appearing in various memoranda. I will also reference Attachments to the CARC report that pertain more specifically to the topic being discussed. I regret not having adequate time to develop final statements directed to the many issues.

Comments

p. iv, last paragraph: ***I remain of the opinion that brain cholinesterase inhibition for males at the 8000 and 16000 ppm dose levels should not be expressed as a consolidated range of 20-43%, but should show 20% inhibition at 8000 ppm and 43% at 16000 ppm. Presenting the findings in the latter manner, makes less tenable the argument that both dose levels are excessive, i.e. the view that the 8000 ppm dose level was not excessive is more defensible when the distinction is made.*** See Att 22 (8 and 16)

p. v, 2nd paragraph: ***I do not recall the Committee's concluding anything to support introducing the phrase "***which was one-half the MTD". ***Wherein was this determination rendered? In any case, in saying that 1000 ppm in the malaoxon study was one-half the MTD, are you not saying 2000 ppm was an MTD, which is an objective to be realized in such studies, and indeed affirms the acceptability of that dose level? In attempting to***

convince others that 1000 ppm, but not 2000 ppm was even an acceptable dose level (let alone one-half an MTD), you should present mortality and cholinesterase data for <u>both</u> dose levels. According to my inspection of the study DER, mortality at 1000 ppm was 42% (males) versus 29% in control and 44% (females) versus 13% in control (as compared with 53% in males and 49% in females at 2000 ppm as you have indicated), plasma cholinesterase inhibition ranged 74-87% (as compared to the 83-96% range you cite at 2000 ppm), red blood cell cholinesterase inhibition ranged 45-66% (as compared to the 54-66% range you cite at 2000 ppm) and brain cholinesterase inhibition ranged 2-30%, where 30% inhibition was observed in males at term (as compared to the 11-78% range, where inhibition in the range of 78% was seen only at term, as you cite at 2000 ppm). It seems to me the Committee treads a fine line in concluding 1000 ppm to be acceptable (particularly in females). What criteria in terms of mortality and degree of cholinesterase inhibition does the Committee employ to say what is or is not excessive? In my view, 1000 ppm is only a questionably acceptable dose under CARC's approach, and therefore certainly not one-half an MTD (particularly in females). Therefore, what is indeed lacking in this study to be satisfactory for assessing carcinogenicity in the absence of competing toxicity, is a dose level approximating one-half the MTD, namely somewhere between 1000 ppm and the lowest dose tested, 20 ppm, i.e. in the 400-500 ppm range.

p. v, 3d paragraph: The Committee determined that the ***single*** oral ***incidences of squamous cell tumors*** (females at ***100/50,*** 6000 and 12,000 ppm***; males at 100/50 ppm***) and nasal tumors (females at 6000 and 12000 ppm and males at ***6000*** and 12000 ppm) etc. etc.

p. v, last paragraph: ***You should show tumor incidences for all dose groups, so people can see incidences at the lower doses in males were increased, though not achieving statistical significance. The remarkably positive Trend Test is undoubtably contributed to by the dosing-related increases in tumor incidence at the lower dose levels. The positive trend across all doses means something, statistically.*** See Att 22

p. vii, 2nd paragraph: (2) spontaneous nasal tumors are very rare in rats ***and therefore the incidences in this study were considered to be biologically significant, particularly in view of the fact that both tumors seen in females occurred in a region (section 5) of the nasal cavity where there was little other evidence of nasal tissue toxicity.*** The trouble with down playing the importance of the olfactory epithelium tumor in males is that while it arose in the region of the olfactory epithelium as opposed to the respiratory epithelium, both of these epithelia were severly compromised toxicologically, and if a tumor were to develop in the olfactory section it would be an olfactory tumor, while if in the respiratory epithelium, it would be a tumor of the respiratory epithelium. I don't think you would expect to find olfactory tumors in the respiratory epithelium or vice versa under normal circumstances. The point is, the nasal tissues were expressing a tumorigenic response of their own type, but yet concertedly in response to the same insult. It is not appropriate to consider the responses as unrelated. Taken together, they attest to a common experience in the two epithelia. As stated elsewhere in these comments, the olfactory epithelium was so severly affected in this study, that in certain cases the olfactory epithelium was replaced. For the male rat in question (#5040), the histopathology sheet reads as follows: "marked - nasal mucosa (olfactory): epithelium-degeneration; nasal mucosa (olfactory): olfactory epithelium replaced by ciliated and nonciliated columnar epithelial cells" It is possible the tumor in question was a tumor of the replacement tissue, and not a neural tissue tumor.

p. vii, last paragraph: 1) Given the finding of the three exceedingly rare squamous cell tumors in females, across the full dose range, the one observed in males is therefore much more

highly suspect than it would be absent the effects in females. I do not accept that the one in males is to be viewed as incidental. Rather, all four squamous cell tumors identified in dosed animals collectively raise a serious red flag. 2) "For females, however, the incidence of oral squamous cell tumors in this study (***1 at 100/50,*** 1 at 6000 ppm and 1 at 12,000 ppm)....."

p. viii, 1st paragraph: 1) "(1) there was no dose response over a wide range of doses (100/50 to 12,000)" ***Actually, the presence of a squamous cell papilloma of the palate at 6000 ppm and a squamous cell carcinoma of the palate at 12,000 ppm constitutes some evidence of a dose response as evidenced in terms of increased severity or progression of the lesion;*** 2) ***Add at end of paragraph that a total of 8 (eight) exceedingly rare tumors (4 nasal, 4 oral) were identified in dosed groups versus none in the control in an assessment of nasal tissues. The oral tumors were detected incidentally from the nasal tissue histopathology assessments, and there has been no oral cavity histopathology assessment to determine whether additional squamous cell tumors occurred in oral tissues. This constitutes a glaring deficiency on the part of both the registrant in not taking responsibility once these tumors were identified, and the Agency in not requiring the work be done.***

p. viii, 2nd paragraph: "There is, however, no evidence to either support or refute this supposition", ***and to that extent the study is unacceptable in males to address this tumor type.*** Att 25

p. ix, 3d paragraph: 1) (ii) the presence of a few rare tumors (oral palate mucosa - ***male*** and female....". ***Also, the squamous cell papilloma in question among males occurred at a dose considered acceptable, i.e. not excessive.*** 2 (iv) malaoxon was positive for leukemia.

p. 4, 4th paragraph: Concerning results of carcinogenicity testing in mice: See Atts 1, 4, 5, 18, 22 (8 and 16), 25

p. 9, last paragraph: "For adenomas at the lower two doses of 100 (15%) and 800 ppm (13%), there was no statistically significant ....." ***In my comments (Attachment 22, p. 151) to the February 2, 2000 draft on this particular subject, I noted in reference to combined tumor incidences that: "To the contrary, the 800 ppm group in my view evidences a clear dose trend with respect to the 8000 and 16000 ppm groups, i.e. control (7%), 800 ppm (16%), 8000 ppm (27%) and 16000 ppm (96%). The increase at 100 ppm (19%) is anomalous and suggestive of a different mechanism, involving more carcinoma."***

p. 10, 2nd paragraph: ***Concerning the inexplicable contrast in tumorigenic responses among females in the two malathion carcinogenicity studies in mice, one might thing CARC would endorse some further pursuit of an explanation.***

p. 10, footnote 3: ***See my response in Attachment 25 to the views expressed in Attachment 23 addressing my comments.***

p. 12, last paragraph: ***I would emphasized the views I have expressed (Atts 1, 12) as to the use of cholinesterase inhibition in discounting dose levels as excessive.***

p. 14, 2nd paragraph: ***In reference to footnote 4, see my response in Attachment 25 to the views expressed in Attachment 23 addressing my comments.***

p. 15, 4th paragraph: "On 14 and 15-March-2000, Cheminova, Inc. ***sponsored a re-***

evaluation of the female liver slides, performed by EPL.

p. 18, top of page: ***In reference to Dr. Copley's assessment of my comments, see Attachment 25 for my responses. Also, in reference to assessments of tumorigenic responses of nasal and oral tissues, see Attachments 13, 14, 15, 19, 20, 22 (8 and 16)***

p. 18, last paragraph: "The biological significance of the adenoma of the olfactory epithelium (6000 ppm male) is unknown since it is from a different cell of origin and this type of tumor (esthesionneural epithelial neoplasm) should not be combined with other tumors of the respiratory nasal cavity." ***The trouble with down playing the importance of the olfactory epithelium tumor in males is that while it arises from the olfactory epithelium as opposed to the respiratory epithelium, both of these epithelia were compromised toxicologically, and if a tumor were to develop in the olfactory section it would be an olfactory tumor, while if in the respiratory epithelium, it would be a tumor of the respiratory epithelium. I don't think you would expect to find olfactory tumors in the respiratory epithelium or vice versa. The point is, the nasal tissues were experiencing a tumorigenic response of their own type, but yet concertedly in response to the same insult. It is not appropriate to consider these responses as unrelated. Taken together, they attest to a common experience in the two epithelia in response to exposure to this chemical. I should also add, that this naming of the olfactory tumor "esthesioneural epithelial neoplasm", does not come out of the study report, i.e. is not terminology employed by the study pathologist. So I am not certain it is the correct terminology that would necessarily apply to the lesion in question. I say this because, as I recall, from the malathion study, nasal epithelial compromises were so severe that in many cases there was complete ablation of the olfactory epithelium, followed by continuous replacement with another tissue type. I am not sure the diagnosis of this lesion would permit application of the indicated terminology. This needs to be checked further, but the press of time does not permit it at this time, so I hesitate to say anything more.***

p. 19, 1st paragraph: ***In reference to footnote 5, see my response in Attachment 25, to the views expressed in Attachment 23 addressing my comments.***

p. 19, 2nd paragraph: ***You should not remove the phrase ...were considered to be biologically significant. Clearly these tumors are biologically significant, given their rarity and the other histopathology in nasal tissues in this study.***

p. 20, 2nd paragraph: ***The finding of three squamous cell tumors in females renders the one in males much more suspect. The registrant and Agency alike should feel duty bound, in the interest of the public health, to pursue a complete oral cavity/oropharyngeal histopathology evaluation of this study, before walking away from it.***

p. 20, 4th paragraph: ***In reference to footnote 6, see my response in Attachment 25, to the views expressed in Attachment 23 addressing my comments.***

p. 20, final paragraph: ***In reference to the interpretation of tumorigenic data of the thyroid, both types, see Attachments 10, 15, 17, 18, 21, 22(8 and 16)***

p. 21, 1st paragraph: ***A positive trend test (p = 0.035) is a positive dose-response. I find in general that while CARC routinely reports the p-value for trend, rarely speaks to its relevance in the interpretation. There is a dose-response trend seen in the data, even in spite of excessive toxicity at 6000 ppm, which suggests to me the real possibility of a more***

remarkable effect at a dose where there is less toxicity somewhat below the 6000 ppm dose level, and this one reason why a dose level as low as 500 ppm cannot be accepted insofar as the study is considered negative in males. This argument is even more compelling in the case of c-cell tumorigenic findings. In reference to footnote 7, see my response in attachment 25, to the views expressed in Attachment 23, addressing my comments.

p. 23, 1st paragraph: "The Committee noted that at 6000 ppm there were still 43 rats considered to be at risk (alive after the first occurrence of carcinoma) which was considered to be an adequate number for evaluation." ***As I indicated at the meeting, a) the 43 rats in question are headed for an early death, and will not likely be <u>as</u> (i.e. for as long) at risk for this reason versus controls and lower dose group animals; b) competing toxicity obtains while the animals live; c) the one incident of carcinoma occurring at such time as to leave 43 at risk may have been spurious, and certainly one such incident is not sufficient to hang your hat on. Furthermore you speak of the fact that although excessive mortality was seen in female rats at the top dose (64% at 12000 ppm) liver tumors were seen at this dose. Who knows what the response might have been had the animals survived. Mortality and competing toxicity may have <u>compromised</u> full expression of liver tumors. You might also have cited leukemia incidences in males as illustrating the compromising effects of excessive doses, where in this study incidences were: 23, 16, 24, 18 and 1 per group at the 0, 100/50, 500, 6000 and 12000 ppm dose levels, respectively. Expression at 12000 ppm was ablated, and I dare say, the finding at 6000 ppm may have been eroded, such that leukemia cannot be claimed as adequately tested at doses of only up to 500 ppm. In any case, each tumor type has its own story. Along these lines, I would refer to Attachment 18 as a principle source of rationale for my dissenting opinion as to the usefulness of findings at excessive doses.***

p. 23, 3d paragraph: ***You should introduce a correction at this point to say, that in contrast to the previous minutes, our consulting pathologist at the last meeting affirmed that c-cell adenomas and carcinomas are easy to distinguish from one another. A balanced approach should compel a comment to the effect that thyroid c-cell tumors are a tumor type of concern in the earlier NCI studies.***

p. 23, last paragraph: ***I would affirm here, that to the extent the study is considered negative across the 0-500 ppm dose range, the study is unacceptable in male rats. In reference to footnote 9, see my response in Attachment 25, to the views expressed in Attachment 23, addressing my comments.***

p. 25, 1st paragraph: ***In reference to the pituitary, the origional pathology report identified a total of 9 carcinomas among female rats in dose groups only. In the re-read 5 carcinomas are remaining among females. The text should give some explanation for the change of diagnosis, in terms of anatomic descriptors. Also, historical control incidences should be presented.***

p. 26, last paragraph: ***In reference to interpretation of testicular tumors, my comments are expressed in Attachments: 8, 11, 25***

p. 27, last paragraph: ***I experience concern over whether CARC appreciates or fully understands the statistically significant findings under the Peto test. The literature indicates that incidences as high as 100% for this tumor type occur near term in normal control F344 rats. I am not aware of any evidence that they appear <u>ubiquitously</u> prior to term. CARC's argument is that because the treated animals died early, there may have***

been early observation of the tumor. The data are saying that, indeed, this is true, yet to find such tumors ubiquitously so early is uncharacteristic, and therefore related to treatment. The results of the Peto Test lead to the conclusion that dosed group animals are arriving at a ubiquitous expression of this tumor type at higher incidences than expected, statistically significantly so. I should note that back when I was reviewing this data, I discussed the same with Dr. Joseph Haseman, NTP Statistician, who advised me the Peto test was the appropriate test, and should it be positive when performed in the prescribed manner, the results should stand. He even offer to perform the test if I sent him the data, which offer I declined. On the other hand, should we acquiesce that the findings are an artifact of the Peto Test, we are left with the need for mechanistic data to answer the question. The CARC report says "there was no serial sacrifice to determine latency". Mechanistic data is the duty of the registrant to provide if he wishes to discount a positive finding. In my view, it is inappropriate for CARC to note the absence of the needed data, and then conclude the findings are not real in the fact of the needed data. So regardless of which argument one uses to skirt these findings, they remain a positive finding across all doses, or certainly the top three, in this study.

p. 28, top of page: ***In reference to footnote 10, see my response in Attachment 25, to the views expressed in Attachment 23, addressing my comments.***

p. 28, 1st paragraph: ***In reference to interpretation of leukemia data, see Attachments 7, 9, 18, 22 (16), 25***

pp. 28-30, entire text: ***The data presented in table 16a indicate a statistically significant increase in MCL among females, plus an increase bordering on significance (p = 0.059) at the 500 ppm dose level. As suggested for other tumor types, a different machanism of tumor induction may occur at the extreme ends of the dose range (100/50 to 12000 ppm). As discussed in Attachment 7, Huff et al noted a statistically significant increase by life-table analysis in males at the low dose level (2000 ppm) in the older NCI study, but discounted this on the grounds that life table analysis should not be used because leukemia was not a cause of death (death attributed to "chemical toxicity", whatever that means). Yet, in the current F344 rat study, leukemia was clearly a cause of death, and therefore I would submit was likely so in the former study. Leukemia was a finding among male rats in the more recent malaoxon study. So there are indications of a leukemia concern, but much has already been said about this. As to the question about increased percentages of male rats harboring MCL, dying of leukemia in a dose related manner, the data are clear. In my discussions about this observation with Dr. Robert Maronpot, NTP pathologist, I was impressed by his affirmation of such findings as indicative of compound-related progression of the response, and hence, evidence of carcinogenicity under the OSTP (1985) definition of carcinogen. I am not convinced the rationale presented on p. 29 of the CARC report serves to discount or undermine this evidence of malathion induced progression of the illness to death. According to Dr. Maronpot, progression in the case of leukemia does not rest with a sequence of events such as those in hepatocellular neoplasia (foci of cellular alteration > adenoma > carcinoma), rather leukemia is a malignancy, and progression is indicated by degrees of invasion of such tissues as liver and spleen.***

Thus when a death due to leukemia is diagnosed, that diagnosis rests with estimates of magnitude of invasion, a manifestation of progression.

The opinion was expressed at the CARC meeting that leukemic animals may, as a

consequence, be weakened animals, and more vulnerable to death as a general consequence of the toxicity of the test material, as opposed to suffering a bona fide primary effect of the test material in enhancing the course of the disease. The problem with this argument is two fold: a) this argument denotes a mechanism that would have to be addressed by the registrant to get around this finding; b) it does not serve to explain the findings at the lower doses of 100/50 (especially) and 500 ppm, unless we are to recognize meaningful toxicity at these doses. An addeded concern about this effect is that it extends across all doses, and is another example of manifestation of effects at the lowest dose level. We realize that although 100/50 ppm is a low dose, it was incorporated in these studies (rat and mouse) in search of a NOEL for cholinesterase inhibition, which was a positive finding at 100 ppm in the rat, and nearly so in the mouse, constituting evidence of the manifestation of a biological effect of malathion at this dose level.

p. 30, 1st paragraph: ***In contrast to the claim in 2) of no statistical significance, please examine your own Table 16a, as the comments in general to not serve to address the evidence of progression based on a dose-related increase in MCL animals dying of the illness.***

p. 30, footnote 11: ***I would suggest that footnote #11 refers to item #4 as opposed to #7. In reference to footnote 11, see my response under Attachment 25, to the views expressed in Attachment 23, addressing my comments.***

p. 30, 3d paragraph: "At 500 ppm there was a non-statistical, but probably biologically relevant decrease (in mortality for males) when compared to controls (47% as compared to 33% in controls)." ***This acknowledgement that in effect mortality was increased in males even at the dose level of 500 ppm, compared to no effects in females to doses as high as 6000 ppm, serves to underscore the view expressed by me that <u>to the extent the study is considered negative for carcinogenicity</u>, it is not an acceptable study for males. The F344 rat may be a poor model for evaluating mlathion in male rats at suitably high doses.***

p. 31, footnote 12: ***In reference to footnote 12, see my response under Attachment 25, to the views expressed in Attachment 23, addressing my comments.***

p. 35, last paragraph: ***I continue to have trouble with discounting the leukemia finding at 2000 ppm in the face of the positive trend and pairwise comparison. I refer to Attachment 18 as presenting rationale for not discounting the finding, based primarily upon the fact that CARC has not demonstrated that the finding was due to toxicity as opposed to tumorigenicity of the test material. Also the nearly positive finding at 1000 ppm (p = 0.07), where the incidence of leukemia as shown was actually higher than at 2000 ppm, and which likely contributes to the positive trend, should not be ignored as constituting at least biological evidence of a positive effect. Furthermore, what would become of the Peto Test findings for the 1000 ppm group if the high dose group were eliminated?***

p. 36, 2nd paragraph: ***Mortality in the malaoxon study at 1000 ppm should be included in this paragraph. Also, since you say that: "There was <u>severe</u> (emphasis added) inhibition of cholinesterase activity for all three compartments (plasma, RBC and brain) in both sexes at 1000 and 2000 ppm at various time points during treatment compared to controls.", what criteria pertaining to the use of cholinesterase data did CARC employ to conclude the 1000 ppm dose level to be acceptable in the face of this degree of cholinesterase inhibition. This serves to underscore my concerns expressed elsewhere over the use of cholinesterase inhibition to conclude dosing to be excessive, and in so***

doing, discounting positive tumorigenic findings.

pp. 36-38; 41-44, concerning Mutagenicity: ***The subject of the mutagenicity seems to have been remarkable revised. My response to this is to recommend an External Peer Review of the subject in the hope of obtaining a more definitive understanding as to interpretation, and whether we have adequate data to reach a definitive conclusion. I would desire more outside expertise directed to the question of whether additional mutagenicity testing is indicated.***

p. 44, next to last paragraph: "It could not be determined whether nasal and oral tumors in female Fischer 344 rats and one nasal tumor in ***each of two male rats and one oral tumor*** in a male rat were treatment-related or due to random occurrence." ***This has all been discussed previously, but I should emphasize here the study has revealed via <u>nasal tissue</u> histopathology, a total of eight exceedingly rare tumors occurring only in dosed groups, four in nasal tissues and four identified incidentally in oral tissues that happened to be located on nasal tissue slides. One way to help in the determination would be to require a full histopathology assessment of oral cavity tissues, not previously performed, either in the origional study, or the subsequent re-examination of nasal tissues. I am concerned that the quoted sentence presented above down plays the number of exceedingly rare tumors observed from eight to six.***

pp. 46- 49, entire text: ***This appears to be a re-iteration of that which has been claimed earlier in the report to which my comments were adequately presented.***

p. 50, footnote 13: ***In reference to footnote 13, see my response under Attachment 25, to the views expressed in Attachment 23, addressing my comments.***

Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist
Toxicology Branch/HED

ATTACHMENT 25

Date: March 30, 2000

MEMORANDUM

SUBJECT: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion

PC Code: 057701
DP Barcode: D264236
Submission #: S529758

FROM: Marion Copley, D.V.M., D.A.B.T.
CARC Member
Registration Action Branch 1
Health Effects Division (7509C)

TO: William Burnam, Committee Chair
Carcinogen Assessment Review Committee
Science Analysis Branch
Health Effects Division (7509C)

Margaret Stasikowski, Director
Health Effects Division (7509C)

As you requested, I have reviewed the 14 areas of concern expressed in Dr. Dementi's memoranda. In summary, I found two issues that I recommend be considered by the Carcinogen Assessment Review Committee (CARC) and several areas that need editorial correction or clarification.

This memorandum responds to those concerns raised by Dr. Dementi about the cancer assessment of malathion. Dr. Dementi expressed these concerns in 22 memoranda dating from November 26, 1997 to February 9, 2000, all cited in and attached to the Carcinogen Assessment Review Committee Report of February 2, 2000 (called CARC Report in this memorandum), and in summary memoranda to John Carley, dated January 27, 2000, and February 3, 2000.

This memorandum clarifies the CARC position on the 14 issues raised by Dr. Dementi, but does not in itself revise the CARC position as stated in its report. Areas identified as needing further evaluation or other clarification by the CARC will be considered at the April 6, 2000 CARC meeting. Any inconsistencies or errors identified in the CARC Report will also be corrected at that time.

References 21 and 22 identify items in the CARC Report that Dr. Dementi considered to be either factually incorrect, unclear or inconsistent. This memorandum addresses concerns expressed in reference 21. However, the response to reference 22, will be completed subsequent to the completion of this memorandum due to the large number of comments (about 50). These comments, as noted earlier, primarily involve errors or inconsistencies in the CARC Report. Any scientific issues should have been identified in the 14 areas of concern and responded to in this memorandum.

It should be noted that my references to female rat liver tumors are based on the data as it existed as of the February 22, 2000 CARC Report. Chiminova has recently submitted revised tumor incidences for these tumors based on a PWG evaluation. The new submission will be discussed at the CARC meeting currently scheduled for April 6, 2000. Therefore, my comments regarding female rat liver tumors may not apply if the new values are accepted. This applies primarily to items 8 and 14.

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1) Mouse liver tumors (ref. 1, 4, 5, 8, 16, 17, 18)

a) Dementi Summary: There was a positive liver tumorigenic response across all doses, i.e., no NOEL for males, and a positive response at the top two doses in females. The finding extending to the lowest dose in males, not unlike the liver tumorigenic response in the female rat in this respect, should be regarded as of particular concern.

Response:

These comments consider adenomas, carcinomas and the combined adenoma/carcinoma response in male mice. The combined response was driven by the adenomas. The carcinomas had no dose response and were not statistically significant either by pair-wise comparison or by trend.

The two high doses (8000 and 16,000 ppm) (CARC report; Table 2) were considered to be positive (adenomas and combined) for a tumorigenic response. Although this was confounded by excessive toxicity at these doses, the tumor response was not "discounted."
For adenomas at the lower two doses of 100 (15%) and 800 ppm (13%), there was no statistical significance by pair-wise comparison, no dose related increase, and the values were within the historical control range of 14 to 22%.[14] The tumor response was actually at the low end of the range.

The concurrent controls were well below the historical control range (7% as compared to 14%). This supported the conclusion that, what could have been interpreted as a treatment-related increase of tumors at the two low doses, was actually due to an unusually low control incidence.

When compared to the historical control data, the incidence of carcinomas at the low dose of 100 ppm (7%) was only slightly outside the range (0 to 6%), and the incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range. In the five historical control studies, the incidences of liver carcinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in an another study (6.4%).
Tumors (adenomas) occurred in the control animals.

The tumor incidence in female rats at 100 and 500 ppm, was considered to be suggestive evidence of carcinogenicity and could not be discounted for the following reasons:
Although the incidences were not statistically significant, they were above the historical control mean. There were no tumors in the concurrent control group. This tumor has a low historical control incidence in female rats. There was a positive response in at a non-toxic dose (6000 ppm). Therefore the CARC was concerned about the low dose response in the female rats.

There are several differences in the low dose response between the male mice (noted above) and the female rats. 1) This is a common tumor in male mice while it is uncommon in female rats; 2) the incidences in mice at the low doses were at the low end of the historical control range while they were above the historical control mean; 3) There were tumors in the controls male mice (although at an unusually low incidence), but no tumors at all in the female rat control group.

[14] combined values and the means were not available

Although the CARC agreed with Dr. Dementi's comment that there was a positive response in the two high doses in both male and female mice, for the reasons delineated above; the CARC did not consider there to be a tumorigenic response at the two low doses in male mice. In addition, although the CARC considered the effects at the high doses to be positive in males, they also considered these doses to be excessive due to marked cholinesterase inhibition. There was also decreased absolute body weights ranging from 9.7 to 20 % depending on sex and dose. Based on this toxicity, the CARC felt that positive tumor data at the two high doses, when considered with the rest of the data base was supportive of (rather than evidence for) the qualitative determination of malathion as a "likely human carcinogen." The data at these high doses was not discarded.

Table 2. Male Mice: PWG Re-read, 1998 - Liver Tumor Rates[+] and Exact Trend and Fisher's Exact Test Results

Tumor Type	0 ppm	100 ppm	800 ppm	8000 ppm	16,000 ppm
Adenomas % p=	4/54 7 **0.000****	8[a]/54 15 0.180	7/55 13 0.274	**14[a] /55** **25** **0.0103*****	**49[a] /51** **96** **0.000****
Carcinomas % p=	0/54 0 0.128	4/54 7 0.059	2[b]/55 5 0.252	2/55 4 0.252	0/51 0 1.0
Combined % p==	4/54 7 **0.000****	10[c]/54 19 0.075	9/55 16 0.125	**15[d] /55** **27** **0.006*****	**49/51** **96** **0.000****

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals (Statistical Analysis, Brunsman, 2/16/99).

[a] First liver adenoma observed at week 53, dose 16,000 ppm, in an interim sacrifice animals. Subsequent liver adenomas observed at week 79, simultaneously in the 100, 8000 and 16,000 ppm dose groups, in terminal sacrifice animals.

[b] First liver carcinoma observed at week 65, dose 800 ppm.

[c] Two animals in the 100 ppm dose group had both an adenoma and a carcinoma.

[d] One animal in the 8000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One male in the 16,000 ppm dose group of the interim sacrifice group had a liver adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then p <0.05; If **, then p <0.01

1) Mouse liver tumors (continued)

b) Dementi Summary: The CARC should not leave unexplained, the more remarkable liver tumorigenic responses, particularly in females, that were observed in the more recent mouse study as opposed to those in the earlier NCI study. This is of particular concern since the new study was designed to replicate at the top two doses.

Response:
As stated in the CARC Report:

> "In the 1978 NCI study with B6C3F1 mice, liver tumors (11 carcinomas and 6 adenomas) were seen in 17 of 55 male mice at the highest dose tested (16,000 ppm); there was no carcinogenic response in female mice. Also in the NCI study, among females, the combined adenomas/carcinomas incidences were 0% at 8000 ppm and 4% at 16,000 ppm in contrast to the present study where the tumor incidences in females were 19% at 8000 ppm and 84% at 16,000 ppm. The Committee noted that the tumor responses in the present study at the same dose levels were more pronounced than those seen in the NCI study."

Other than making the observation that the more recent mouse study has a more pronounced response, the CARC was unable to make any further observations. Given the information the Committee had about both studies, anything further would be speculation and would not add to the risk assessment process.

2) Thyroid c-cell tumorigenic response in the male rat (ref. 10, 17)

Dementi Summary: This finding is positive among male rats across the 0-500 ppm dose range, and cannot be discounted as CARC has done by findings at higher "excessive" doses, lest the study be considered unacceptable for evaluation of this tumorigenic response. Findings at low doses should be of particular concern and discounted only by the most persuasive forms of evidence.

Response:

(CARC report; Table 10a) Following discussion with the consulting veterinary pathologist, the incidences of combined thyroid c-cell tumors were determined to be the most appropriate tumor values for the final evaluation due to the difficulty in distinguishing the individual tumor types (i.e., adenomas vs. carcinomas)[15]. It is true that there is statistical significance by pair-wise comparison for thyroid c-cell carcinomas at the 500 ppm (both with and with out considering the 2 high doses) (2%, 4%, 13%**, 5%, 0%, for controls to high dose). The CARC did consider the possibility that the excessive mortality in males at the top doses (74% at 6000 ppm and 100% at 12,000 ppm) may have compromised the expression of this tumor at these (higher) doses. However, the Committee noted that at 6000 ppm there were still 43 rats considered to be at risk (alive after the first occurrence of carcinoma) which was considered to be an adequate number for evaluation. Therefore, there was no dose response and the increase at 500 ppm was considered to be due to variation rather than malathion. For the combined tumors, there was no statistically significant trend, pair-wise significance, or dose-response at any dose level, either when all dose groups were included or when the top two doses were excluded from the analyses. Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats. Therefore, the Committee did not agree with Dr. Dementi, and considered that the thyroid c-cell tumors were not attributable to treatment.

[15] Also see Reference: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

Table 10a. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Including All Dose Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
Adenomas p=	13/53 (25%) 0.326	14/54 (26%) 0.461	10/50 (20%) -	6/50 (12%) -	4[a] /35 (11%) 0.242
Carcinomas p=	1/51 (2%) 0.556	2/50 (4%) 0.310	6[b] /45(13%) **0.012**[*]	2/43 (5%) 0.178	0/9 (0%) -
Combined p=	14/53 (25%) 0.430	16/54 (30%) 0.389	14[c] /50(28%) 0.403	8/50 (16%) -	4/35 (11%) 0.242

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 5/3/99).

[a] First thyroid c-cell adenoma observed at week 81, dose 12,000 ppm.

[b] First thyroid c-cell carcinoma observed at week 90, dose 500 ppm.

[c] Two animals in the 500 ppm had both an adenoma and a carcinoma.

3) Thyroid follicular cell tumors (ref. 16, 17, 18)

Dementi Summary: "The competing toxicity and increased mortality among male rats at 6000 ppm and 12,000 ppm (dose levels considered as excessive by CARC) may have *dampened or compromised* full expression of a tumorigenic response at these higher doses already evident in the existing data set, i.e. a positive dose trend ($p = 0.035$) and a nearly positive ($p = 0.077$) pair-wise comparison for the 6000 ppm dose group. I challenge, therefore, CARC's conclusion that the study can be accepted as a negative study for this tumorigenic response. In my view (not stated as such previously, though evident in the reasoning) this tumorigenic response should be viewed as suggestive evidence of carcinogenicity that cannot be discounted because of the unacceptability of the study in male rats at the high dose levels, which CARC itself has called excessive. This is a difficult interpretation which I feel merits an external review."

Response:

(CARC Report; Table 9) The Committee concluded that the thyroid follicular cell tumors were not treatment-related since there was neither a pair-wise significance nor a dose-response relationship for any thyroid follicular cell tumor type (i.e., adenomas, carcinomas or combined adenomas/carcinomas); only a trend was seen for the combined tumors. The argument presented by Dr. Dementi that "competing toxicity … may have dampened or compromised full expression of a tumorigenic response …" is speculative and in this case, the Committee felt that it was inappropriate to speculate what would have happened if mortality wasn't so high at the two high doses.

In addition, although the CARC considered the 6000 and 12,000 ppm dietary concentrations to be excessive for male rats based on mortality and cholinesterase inhibition in all three compartments, the 500 ppm concentration was considered **adequate** (not too low—inadequate) to evaluate carcinogenicity. (For additional details see item 13.) Therefore, the 500 ppm dose was considered to be appropriate for use when evaluating the carcinogenic potential of malathion in the male rat—without requiring any intermediate doses or a new study.

It should be noted that **I recommend** revising the executive summary and weight of evidence sections of the CARC Report to include a statement consistent with what was said for the male rat liver tumors: "**Although there was no evidence of these tumors in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.**" This statement would acknowledge Dr. Dementi's assertion. However, the CARC did not think that it would be appropriate to suggest that there would have been more tumors if a dose in between 500 and 6000 ppm was tested.

Table 9. Male Rat: Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
Adenomas	2/55	1/54	1/51	4/51	4[a]/43
(%)	4	2	2	8	9
p=	0.063	-	-	0.150	0.378
Carcinomas	0/42	0/45	2/41	2[b] /26	0/0
(%)	0	0	5	8	0
p=	0.196	-	0.085	0.162	-
Combined	2/55	1/54	3/51	6/51	4/43
(%)	4	2	6	12	9
p=	**0.035**[*]	-	0.321	0.077	0.160

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/97).

[a] First thyroid follicular cell adenoma observed at week 76, dose 12,000 ppm.

[b] First thyroid follicular cell carcinoma observed at week 100, dose 6000 ppm.

Note: Interim sacrifice and accidental death animals were not included in this analysis. There were no thyroid follicular cell tumors in any of the interim sacrifice or accidental death animals.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

4) Leukemia in the rat: Interpretation of evidence under OSTP (1985)'s definition of carcinogen (ref. 7, 9, 17)

Dementi Summary: "Evidence of a dose related increased incidence of mortality attributed to leukemia ***among male rats diagnosed with leukemia*** constitutes positive evidence of carcinogenicity under the second aspect of OSTP's definition of carcinogen, namely, '… or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen.' (pp. 10410-10415). I contend the dose-related increased mortality (where mortality itself indicates a more advanced stage) is evidence of a dose-related increased rate of development of leukemia. It could be argued that rats harboring leukemia are simply more susceptible to early death due to the increasing secondary toxicologic burden of the test material, but to confirm that possibility and to discount the possibility of a direct compound effect in development of the response, the mechanism would need to be established. I am not aware CARC has provided a rational response to this issue."

Dr Dementi elaborated on this in Ref. 9, noting that leukemia and nephropathy are the primary causes of death in this study. "The number of male rats among 55 rats per group diagnosed with leukemia (death due to leukemia) were 23(7), 16(7), 24(14), 18(13) and 1(1) for the control, 100/50, 500, 6000 and 12,000 ppm groups, respectively. Hence, among rats diagnosed with leukemia, the percentages dying with leukemia were: 7/23 (30%), 7/16 (44%), 14/24 (58%), 13/18 (72%) and 1/1 (100%), in the same respective order." Dr. Dementi further observed that the rate of rats dying due to nephropathy also increased with dose to 47 out of 55 at the high dose. Therefore, he proposed that the decreased expression of leukemia, at least the high dose, is due to competing toxicity from nephropathy.

Response:

(CARC Report; Table 16) The CARC examined this endpoint at several meetings (OCT-15, 1997, FEB-24-1999, JUN-23-1999). The Committee first evaluated the mononuclear cell leukemia (MCL) at the October 15, 1997 meeting, and concluded that the occurrence of this tumor type in female rats was not attributable to treatment because there was no statistical significance at any dose level and the incidences were within in the historical control range of the testing laboratory (15 to 36%). Subsequently, at the February 24, 1999 meeting, the Committee determined that additional statistical analysis using Peto's prevalence test was needed to more accurately evaluate the significance of this tumor type in male rats. Results of this analysis—presented below (CARC Report; Table 16)—were evaluated at the June 23, 1999 CARC meeting. The Committee concluded at that time, that MCL in male and female rats was not treatment related based on: 1) the lack of statistical significance at any dose level, 2) absence of a dose-response relationship, and 3) the incidences were within the historical control range of the testing laboratory (15 to 36%). Additionally, the CARC Report noted that MCL was not seen in three strains of rats: the Osborne-Mendel (1978 NCI-malathion); Sprague-Dawley (1980-FDRL-malathion); and F344 (1979, NCI-malaoxon and the 1996 malaoxon studies). **I recommend** that the following disclaimer be added to this section of the CARC report, "However, the results of the old studies should be used with caution to support or refute any results due to inherent problems in these studies."

Based on review of the minutes for meeting dated June 23, 1999 (printout from the white board), it appears that the issue of "**percent of leukemic animals dying from MCL**," while in the background package given to the CARC members for review, was inadvertently not discussed at the meeting.

I recommend that the CARC reconsider this subject, based on the information presented by Dr. Dementi (Table A, taken from the DER and ref. 9). It appears that there may be evidence of increased severity—as evidenced by the increased percentage of leukemic animals dying due to MCL—with the exception of the high dose. While there is no supporting evidence that competing toxicity from nephropathy is responsible for the decrease in MCL at the high dose, the expression of MCL is extremely low considering that the number of animals considered to be at risk is 52 (alive at time of first occurrence of MCL). This lesion is considered fatal in the more advanced stages of severity.

This may be consistent with the SAP comment (below) that the severity could be increased due to chemical exposure. Since severity was not staged by the study pathologist, the CARC needs to determine if it is supported scientifically to use the incidence of these tumors as the cause of death (determined by the study pathologist) as an indicator of the increased severity at the later stages.

The Scientific Advisory Panel report, "A Set of Scientific Issues Being Considered by the Agency in Connection with DDVP (Dichlorvos) Risk Issues" addressed the use of MCL in cancer risk assessment. "There is an emerging view based on cumulative experience by some toxicologic pathologists that mononuclear cell leukemia in the Fischer rat may be a unique type of cancer and not induced *de novo* by compound administration.…There is compelling evidence to disregard MCL, in the Fischer rat. MCL is one of the most common background tumor types in this strain, and has been referred to as Fischer rat leukemia. Other rat strains and mice do not develop MCL, and there is no human correlate to this disease. Additionally, chemically-related increases in MCL exhibit advanced severity grades for this lesion in treated rats compared to controls."

The relevance of this increase—if it is determined to be real—to human risk assessment is questionable according to the SAP and as presented in a new review of MCL (ref. 26). The DDVP CARC Report #6 (1-MAR-2000) indicated that: 1) MCL is common in the Fischer rat and, in the males …, 2) The tumor type does seem to be found mainly in this Fischer strain and does not appear to be similar to leukemia in humans (adults or children). The CARC concluded that, "while all of this information somewhat lessened our concern, the MCL could not be totally dismissed as not being relevant to humans."

Table 16. Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12,000 ppm
Male	23/55	16/55	24/55	17/53	1[a]/52
(%)	42	29	44	32	2
p=	-	-	0.463	-	-
Female	9/55	**18/55**	15/55	13/54	10[b]/55
(%)	16	**33**	27	24	18
p=	0.917	**0.025**[*]	0.059	0.181	0.670

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/97 & 5/3/99).
[a] First mononuclear cell leukemia observed in a males at week 64, dose 12,000 ppm.
[b] First mononuclear cell leukemia observed in a female at week 47, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no mononuclear cell leukemia in any of the interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

Table A. Male rats (Fischer 344) with MCL that died from MCL

DOSE	control	100/50 ppm	500 ppm	6,000 ppm	12,000 ppm
MCL as cause of death/# with MCL	7/23	7/16	14/24	13/18	1/1
% animals with MCL, dying from MCL	30	44	58	72	100

Table taken from the DER (MRID 43942901)
MCL - mononuclear cell leukemia

5) Interstitial cell testicular tumor in the rat (ref. 8, 11, 16, 17)

Dementi Summary: "Statistical significance of this tumorigenic response was positive across all four doses as presented in the study report, and was positive across the top three doses as analyzed by the Peto test within HED. I accept these assessments as showing a dosing related higher incidence than expected of this tumorigenic response, and hence, as a positive carcinogenic effect by recognized definitions of a carcinogen. In my view, the Peto test, as required by the CARC, was conducted in the prescribed manner by HED's statistician, and was positive. I am not satisfied with CARC's rationale (absent mechanistic data) for discounting this response, and would desire another expert opinion."

Response:

(CARC Report; Table 15) At the October 8, 1997 CARC meeting, the Committee determined that male rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 12,000 ppm dose group with the controls for the interstitial cell tumor, both at $p < 0.01$—using the Peto's Prevalence Analyses protocol. There were also significant differences in the pair-wise comparisons of the 500 ppm and 6000 ppm dose groups with the controls for this tumor type, both at $p < 0.05$. Statistical analyses of this tumor in the study report indicated that the increases in testicular tumors were statistically significant at all dose levels. Statistical analysis by HED obtained essentially the same results, except for the low dose group which did not show pair-wise significance. However, statistical evaluations should not be considered to be the final word without any consideration of the biological relevance of the data. For this tumor type, the historical spontaneous occurrence often approaches 100% by the end of a study. Therefore, in spite of the above statistical evidence, the Committee concluded—and I agree—that, contrary to Dr. Dementi's opinion, the testicular tumors should not be considered treatment related since: 1) this non-lethal tumor was observed in nearly 100% of male rats including controls; 2) the apparent statistical significance of the tumor incidence at 6000 and 12,000 ppm groups [***Note***: both doses were determined to be excessive in males] could be attributed to the high mortality at these doses—resulting in earlier observation of the tumor—and significance was considered to be an artifact of the Peto's Prevalence Analyses protocol; 3) sufficient data were not available to determine if there was a decrease in the latency period [i.e., there was no serial sacrifice to determine latency]; and 4) this tumor type is not useful in overall evaluation since its occurrence is similar at all dose levels.

Table 15. Male Rat: Testes Interstitial Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12,000 ppm
Interstitial cell tumor	52/55	52/55	53/55	52/53	53 [a] /54
(%)	95	95	96	98	98
p=	**0.000**	-	**0.037**	**0.032**	**0.004****
	**		*	*	

[+] = Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/1997).

[a] First testicular tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testicular tumor not in an interim sacrifice or accidental death animal observed at week 64, dose 12,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. Two animals in the 0 ppm dose group and five animals in the 12,000 ppm dose group of the 54-week interim sacrifice group had this tumor. Two accidental death animals in the 6000 ppm dose group had this tumor.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

6) Rat nasal tissue histopathology and tumorigenic response in the rat (ref. 12, 13, 14 16, 17, 19, 20)

Dementi Summary: "I accept as evidence of carcinogenicity all **four** extremely rare nasal tumors, two in males and two in females, at the top two dose levels. CARC discounts the findings in males, as I understand, because dosing was excessive, but again, as with certain other tumor types, to the extent tumorigenic findings are discounted in high dose groups, the study in my view is unacceptable in males. The issue is complicated by evidence of nasal histopathology in the long term combined chronic toxicity/carcinogenicity studies in the F344 rat for both malathion and malaoxon, and in the dose range-finding and subchronic inhalation studies of malathion in the rat. While a new inhalation study is being required, I am not satisfied that CARC has an adequate interim handle on risks posed with respect to the nasal mucosa, particularly by the inhalation route of exposure. Nasal tissue vulnerability is an important and unresolved issue at this time."

Response:

I have identified several inconsistencies in the CARC Report regarding the male nasal tumors and their contribution to the weight of the evidence. These need to be corrected when the report is revised following the April CARC meeting. The CARC report should be corrected to consistently say that, 1) the nasal tumor in the high dose males is supportive (but not strong evidence by itself) evidence, 2) the biological significance of the olfactory epithelial tumor is unknown since it is from a different cell of origin and these types of tumor (esthesioneural epithelial neoplasms) should not be combined with other tumors of the respiratory nasal cavity[16].

Based on the data currently in house, the CARC can not develop (as requested by Dr. Dementi) an "interim handle on risks posed with respect to the nasal mucosa, particularly by the inhalation route of exposure." It is hoped that the required 90 day inhalation study will shed light on this issue. In the absence of any other information, The CARC stated, "In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996) [ref. 25], the Committee classified malathion as a **"likely human carcinogen** by all routes of exposure." This includes the inhalation route. Therefore, in the absence of data, I feel that the CARC is taking the most conservative approach by considering there to be a potential carcinogenic risk by exposure from the inhalation route.

(CARC Report; Table 8) The CARC agrees with Dr. Dementi that the nasal tumors (respiratory adenoma) in the female rats are evidence of carcinogenicity. "**The Committee … concluded that there is evidence of carcinogenicity for malathion in female rats** … which manifested as … tumors of the nasal mucosa at 6000 ppm, although nasal tumors were also seen at 12,000 ppm (a dose considered to be excessive)." The statement "(but not in males)," that was in the previous sentence in the report, will be removed (as noted above) since it is inconsistent with the remainder of the CARC Report. The CARC did not discount the tumor at the female high dose, but felt that more weight should be placed on tumors that occurred at non excessive doses.

There are two different types of tumors in the male—an **adenoma of the olfactory**

[16] *Also see*: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

epithelium at 6000 ppm and an **adenoma of the respiratory epithelium** at 12,000 ppm compared to zero for each in the controls. The CARC considered that the adenoma of the respiratory epithelium added to the concern even though it was at an excessively toxic dose. As noted earlier, these two tumor types should not be combined. Therefore, it can not be determined whether the olfactory epithelial tumor increases our concern or not for nasal tumors in the rat.

Table 8. Neoplastic Findings of the Nasal/Oral Tissues in Rats

TUMOR TYPE	Dose (ppm)				
	0	**100/ 50**	**5 0 0**	**60 00**	**12,0 00**
MALES (No. Examined: 90/dose[a])					
Nasal Olfactory Epithelium **Adenoma**	0	0	0	**1**	0
Nasal Respiratory Epithelium **Adenoma**	0	0	0	0	**1**
Palate, Squamous Cell papilloma	0	1	0	0	0
FEMALES (No. Examined: 90/dose[a])					
Nasal Respiratory Epithelium **Adenoma**	0	0	0	**1**	**1**
Tooth, Alveolus, Squamous Cell Carcinoma	0	1	0	0	0
Palate, Squamous Cell Papilloma	0	0	0	1	0
Palate, Squamous Cell Carcinoma	0	0	0	0	1

[a] This is uncensored data. There were only 55 rats/sex/dose in the 2 year portion of this study, the remainder were sacrificed at 3, 6 or 12 months.

7) Oral cavity assessment for tumorigenic response: Its adequacy and CARC's conclusion regarding squamous cell tumorigenic response (ref. 13, 14, 15, 17, 19, 20)

Dementi Summary: "I contend the **four** extremely rare squamous cell tumors (three in females, one in males) appearing in oral mucosal tissues in the malathion combined chronic toxicity/carcinogenicity study in the rat cannot be discounted as evidence of carcinogenicity. Furthermore, as these tumors were identified in but a partial and inadequate assessment of oral cavity histopathology, there is a greater incumbency to accept these as real until an adequate histopathology assessment of the entire oral cavity tissues has been performed. I have suggested this need for additional histopathology to CARC, and am concerned the registrant did not of his own volition follow-up with a complete oral cavity histopathology assessment once these tumors were found. CARC discounted the oral tumors at one meeting on the grounds these tumors are not as rare as the nasal tumors. However, subsequent follow-up information, in my opinion, demonstrates the squamous cell tumors to be essentially as rare as the nasal tissues in various data bases. I am not aware CARC has responded to the more recent information. I have not been availed of the latest, or final, CARC report."

In ref. 14, Dr, Dementi noted, "In consideration of the fact that the four nasal tumors were considered treatment-related while the four oral tumors were not at the June 23 CARC meeting, toward the end of the meeting a CARC member sought an explanation for this voting disparity. The CARC response was clear, the nasal tumors are very rare, historically, but the oral tumors are not. Yet, this subsequent and closer scrutiny of the NTP data base indicates that squamous cell tumors of the palate are as rare as the nasal tumors. The rationale for the difference in vote does not exist."

Response:

(CARC Report; Table 8, above) The CARC Report concluded that, "Palate tumors were observed at 100/50 ppm (a squamous cell papilloma in 1/90 males), at 6000 ppm (a squamous cell papilloma in 1/90 females) and at 12,000 ppm (a squamous cell carcinoma in 1/90 females). These tumors were not attributed to malathion treatment due to lack of statistical significance, and absence of a dose-response in either sex." The support for this was that they were not as rare as the nasal tumors.

I recommend that this issue be reexamined by the CARC. This is based on the information presented by Dr. Dementi, particularly the new historical control discussion indicating that they are rare (ref. 13). Dr. Dementi makes a valid point that, once the difference in historical controls goes away, the oral tumors have the same pattern of occurrence as the nasal tumors and should be evaluated with the same criteria. Also of concern is the squamous cell tumor of the tooth (alveolus) in a low dose female. This is also an oral squamous cell tumor and should be combined with the other oral squamous cell tumors (phone conversation with Dr. Brenneke, FEB-29-2000, 8:50 AM) resulting in single tumors in the 100/50, 6000 and 12,000 ppm female groups. Therefore, there may be an additional concern for this tumor type in females since there are two groups with tumors in the absence of excess toxicity. In the males however, there is only 1 tumor, and that occurs in the low dose where there are no other oral tumors. Therefore, I feel that it is difficult to attribute any biological significance to the occurrence of a single tumor occurring only at the low dose.

I have some concern that the historical control values as presented by Dr. Dementi are misleading. All squamous cell tumors of the oral cavity should be considered together. The historical control data as presented in ref. 20, are for tumors of the palate and for tumors of

the tooth, independently. The appropriate historical control data should include all animals bearing squamous cell tumors of the oral cavity. The values presented in ref. 14 from the NTP data base, did support Dr. Dementi's contention that these are rare tumors in rats. There were very few squamous cell carcinomas (0/901) and papillomas (2/901) of the oral mucosa (including the palate) in females. Values for male rats were similarly low, 1/904 and 2/901 for carcinomas and papillomas, respectively.

Dr. Dementi also expressed concern that the oral cavity was not routinely examined histologically. In his memorandum to Patricia Moe (SRRD) (ref. 19) he says that, "Dr. Bolte indicated that all cavities, oral cavity included, received postmortem examinations for macroscopic abnormalities, and that the tissues associated with the oral cavity included the lips, gingiva, teeth, buccal mucosa, tongue and hard palate." "Specifically, Dr. Bolte provided assurances that oral cavity tissues in question were examined macroscopically, but he advised that the oral cavity is not a protocol tissue." Therefore, negative findings were not reported and the tissue did not routinely undergo histologic examination. There were no macroscopic lesions observed in the oral cavity, indicating that the oral tumors were all microscopic in size. Oral tissue was only examined incidentally in nasal sections. We do not know how many had slides with incidental negative oral tissue present since this was not reported. As a result, the actual incidences of these tumors in the study may be underestimated. Therefore, **I recommend that the Committee consider the advisability** of asking for a routine microscopic examination of the oral cavity, if it appears it could have an impact on the ultimate weight of evidence and cancer classification.

8) Tumorigenicity (several end points) in low dose groups [Concerted evidence of]. For example, are the low dose hepatocellular tumorigenic responses in the mouse and rat mutually supportive? (ref. 1, 15, 16, 17)

Dementi Summary: "I have expressed concern over certain tumorigenic responses that appear to extend into the low dose range, incorporating in certain cases even the lowest dose, absent a NOEL (e.g. male mouse liver tumors, female rat liver tumors, leukemia in male rats as defined above, extremely rare oral squamous cell tumors, possibly testicular tumors). My concern is whether collectively these speak more strongly of a low dose biological effect, than any standing alone, and whether CARC has adequately addressed this possibility in its assessment."

Response (see last paragraph, page 2):

In response to Dr. Dementi's concern regarding low dose tumors, it should be noted that the CARC Report did not consider there to be a low dose response in any of the tumors, with the exception of liver tumors in the female rat. The issue of oral squamous cell tumors occurring at the low dose in female rats will be revisited. The CARC does consider that the quantitative risk assessment using the female rat takes into account the increase in tumors observed at all doses, including the low dose. CARC Report already expressed concern for one (this may be changed to two) tumor type occurring at the low dose. The CARC considered this in the weight of evidence, and classified malathion as a "likely human carcinogen." Dr. Dementi questions whether the CARC has adequately addressed the possibility that collectively the occurrence of low dose tumors "speak more strongly of a low dose biological effect, than any standing alone. …" I feel that the narrative that accompanies the cancer classification, adequately describes the tumors of concern, noting factors (such as occurrence at either low or excessive doses) which increase or lessen this concern. Therefore, no additional evaluation is required, with the exception of oral tumors in the female rat and MCL in the male rat and how they affect the weight of evidence, if at all.

9) Decisions to discount dose levels as excessive for carcinogenicity assessment based on cholinesterase inhibition (ref. 1, 8, 12, 16)

Dementi Summary: "Inherent in such review would be the precedent for the decision, existence of guidelines, which forms of the enzyme must be inhibited and by how much, and so on. I do not accept the view that cholinesterase inhibition (absent any guidelines or rationale) alone, absent cholinergic clinical signs, can be cited as adequate rationale to discount a dose level in question as excessive, and in so doing discount remarkable tumorigenic findings observed at that dose level. In my view, inadequate rationale has been provided by CARC to justify dismissal of dose levels as excessive, and in so doing precluding testing at high doses (MTD) called for in cancer bioassays."

Response:

I identified an error by omission in the CARC Report executive summary. The report stated "The Committee concluded that in mice, the 800 ppm dose level was adequate to assess the carcinogenic potential of malathion, however, the 8000 and 16,000 ppm doses were excessive based on severe plasma (90 to 95%) and red blood cell (92 to 96%) and marked brain (20 to 43%) cholinesterase inhibition in both sexes." It didn't mention that at these doses, there was also a marked decrease in absolute body weight (throughout the study). This is discussed in the body of the CARC Report and will be added to the executive summary in the revised report.

The CARC considers cholinergic inhibition in conjunction with the rest of the data base for a particular study and chemical. This includes: how many compartments (with particular attention to brain cholinesterase) are effected, the magnitude of response, the presence of clinical signs, changes in body weight and food consumption, mortality as well as the presence of cholinergic signs.

In the Fischer 344 rat malathion cancer study, the doses of 500 ppm in males and 6000 ppm in females were considered adequate to assess the carcinogenic potential of malathion; but 6000 ppm in males was excessive due to increased mortality (74%); and the 12,000 ppm was excessive in both sexes based on the severe inhibition of plasma (89%), red blood cell (52%) and brain (67%) cholinesterase activity in females and increased mortality in males (100%) and females (64%) at this dose. In this case, not only was there evidence of cholinesterase inhibition in all three compartments, there was also increased mortality. This was also true for the rat malaoxon study where the dose level of 1000 ppm was considered adequate to assess the carcinogenic potential of malaoxon, but the 2000 ppm dose was excessive due to increased mortality (53% in males and 49% in females) and severe inhibition of plasma (83-96%), red blood cell (54-66%) and brain (11-78%) cholinesterase activity.

In the mouse malathion cancer study, the 800 ppm dose level was considered adequate to assess the carcinogenic potential of malathion, however, the 8000 and 16,000 ppm doses were considered excessive based on severe plasma (90 to 95%),severe red blood cell (92 to 96%) and marked brain (20 to 43%) cholinesterase inhibition in both sexes. All three compartments were affected and there was almost 100 % inhibition in the first two. The NOAEL for plasma and RBC cholinesterase inhibition in the mouse was 100 ppm, and that for brain cholinesterase inhibition was 800 ppm for both sexes—substantially lower doses than those considered excessive. There was also decreased absolute body weights at 8000 and 16,000 ppm in both sexes, ranging from 14.3-20.0% in males and 9.7-16.1% in females throughout the entire duration of the study. The final body weights for the 8000 and 16,000 ppm groups were between 3 and 7 grams less than controls. Although there was neither

mortality or clinical cholinergic signs of toxicity in the mouse, the presence of marked cholinesterase inhibition and decreased absolute body weights at 8000 and 16,000 ppm in both sexes is supporting evidence of excessive toxicity. Therefore, the tumor results at the high doses in the mouse, while not discounted, should be used with caution.

The EPA Draft Cancer Guidelines from 1996 and 1999 (ref. 25) state that excessive doses may be determined by factors such as: significant toxicity or perturbation of physiological function; reduction in body weight gain of greater than 10% over the lifespan of the animals; and significant increases in mortality from effects other than cancer. Using this reasoning, I feel that excessive cholinesterase inhibition can be considered to be either a perturbation of physiological function or toxicity depending on what else is happening to the animal. It should be noted that using cholinesterase inhibition—or any other endpoint—when establishing that doses are excessive, does not imply an effect (either positive or negative) by that endpoint on the tumor response. It indicates a compromised animal where homeostasis is altered—"… that would confound the interpretation of study results to humans." Whether the response indicates an adequate dose or an excessive dose depends on the magnitude of the response. Although the CARC evaluates the adequacy of dosing on a study by study and often on a dose by dose bases, it strives for consistency across studies and chemicals. The CARC does not—as presented in Dr. Dementi's item 9 summary—"discount a dose level in question as excessive, and in so doing discount remarkable tumorigenic findings observed at that dose level." I feel the CARC adequately considered the issue of what constitutes excessive toxicity.

10) Acceptability of OSTP's (1985) definition of carcinogen, and if considered acceptable, the rigor of its application in CARC's interpretation of the malathion studies. (ref. 5, 8, 9, 11)

Dementi Summary: "The OSTP (White House Office of Science and Technology Policy) definition reads as follows: 'A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen.' (pp. 10414-10415) I have sought from CARC its views as to the veracity of this definition of a carcinogen, but my question has not been acknowledged or addressed. I posed the question because it seemed to me that on certain of the tumorigenic end points, the committee appeared too focused on the first element of the definition (strict statistical treatment of tumor incidence) to the neglect of second element (rate of tumor development). Evidence of enhanced tumor development, including such findings as greater proportions of malignant versus benign tumors, tumor multiplicity, tumor size, decreased tumor latency, etc, may not yield statistical evidence of carcinogenicity, but yet constitute positive evidence of carcinogenicity according to the OSTP definition. If CARC owns this definition, then it should provide more evidence of its utilization in the interpretation of the end points at hand."

Response:

I do not feel that the EPA cancer guidelines (ref. 25) are not in conflict with the OSTP definition. In the 1999 draft they state, "In, general, observation of tumor effects under different circumstances lends support to the significance of the findings for animal carcinogenicity. Significance is a function of the number of factors present and, for a factor such as malignancy, the severity of the observed pathology." The CARC does consider both tumor incidence and enhanced tumor development when evaluating the carcinogenic potential. These factors are considered, in conjunction with the remainder of the data base in the weight of evidence determination. HED has been instructed to use the EPA Draft Guidelines for Carcinogen Risk Assessment (ref. 25) and therefore, should provide evidence that it is following these guidelines, not the OSTP.

For malathion, if the Committee determined that a tumor had significant evidence of increased severity or decreased time for a spontaneous tumor to develop, it would have to take that into consideration in the weight of evidence. The guidelines specify that no one issue should be taken in isolation. In the case of MCL, **I am recommending that the Committee reconsider this issue** to determine whether there is supportable evidence for increased severity or decreased latency in the absence of increased incidence. In most cases, it is difficult to determine whether there is decreased latency since few studies have serial sacrifices.

11) Incorporation of tumorigenic findings or the absence (or reduced incidences) of the same, at doses considered excessive. (ref. 4, 9, 10, 17, 18)

Dementi Summary: "Questioned here is the use of tumorigenic findings, or the absence of the same, in a dose group considered excessive by CARC. A prime example is CARC's use of the top two dose groups (6000 ppm and 12,000 ppm), considered excessive doses by the Committee, for assessing tumorigenicity among male rats, in the combined chronic toxicity/carcinogenicity study in the rat. I contend as improper the discounting of tumorigenic findings of one type at a dose level considered excessive, while utilizing decreased tumorigenic findings of another type in these excessive dose groups to discount positive findings at lower doses considered by the committee to be acceptable. By contrast, I contend that accepting tumorigenic findings at excessive doses is more defensible than accepting as negative a study without findings at excessive doses."

Response:

The following response to Dr. Dementi's concern was taken from a memorandum written by William Burnam (ref. 23). This memorandum was written by Dr. Burnam in response to the OCT-28-1999 memorandum from Dr. Dementi (ref. 17). I have made minor spelling and editing changes to original version.

The problem of setting doses for cancer studies and judging the significance of tumors at excessive doses is one that the CARC and Agency Cancer Risk Assessment Guidelines have been trying to deal with for a long time. This is what our current draft Agency Guidelines (page 2-12, 2-13) state:

> Excessive high dose: If toxicity or mortality is excessive at the high dose, interpretation depends on the finding of tumors or not.
>
> (a) Studies that show tumor effects only at excessive doses may be compromised and may or may not carry weight, depending on the interpretation in the context of other study results and other lines of evidence. Results of such studies, however, are generally not considered suitable for dose-response extrapolation if it is determined that the mode(s) of action underlying the tumorigenic responses at high doses are not operative at lower doses.
>
> (b) Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits.
>
> (c) If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity.

In the malathion example, the CARC has determined that based on dose and tumor response, the liver tumors in mice and rats indicate different interpretations.

In the mouse study, there were liver tumors at the two high doses in both males and females but no increase in liver tumors at the lower doses. The two higher doses were judged by the CARC to be excessive, while the lower doses, where no increases in tumors were seen, were adequate. It should be noted that even though, these two higher doses were excessive in terms of different types of cholinesterase inhibition seen, there were sufficient mice at risk (living long enough) to determine a carcinogenic effect for these tumors and that this

carcinogenic effect was part of the CARC weight of evidence in its determination of a "likely" classification. Since there were no tumors at lower, non-excessive doses, the liver tumors in mice were not used for dose-response extrapolation.

In female rats, a statistically significant increase in liver adenomas and carcinomas was seen at the highest dose—a dose considered excessive by the CARC. However, in contrast to the mouse study, there was also statistical significance at the next to the highest dose and a biologically significant increase at the two low doses when compared to the control. None of these lower doses were considered excessive. The dose response information from this rat study, spanning the doses from excessive to adequate, is the basis for the dose response extrapolation for human risk assessment and contribute heavily to the classification of malathion as "likely."

The treatment of other tumors in the rat uses the same rationale as the CARC did for liver tumors in mice and rats. The combined incidence of adenomas and carcinomas of the male thyroid follicular cell showed a significant trend but no increases by pari-wise analysis. Again, even though the two highest doses were considered excessive, there were sufficient rats at risk to be used in a carcinogenic analysis by Peto's Prevalence Test. The facts that there was only a trend for this combined follicular cell tumor in males was not a major contributor to the CARC's classification decision.

Likewise, with the c-cell thyroid tumor in male rats, there were sufficient rats at risk at the two highest—although excessive—doses, to be used in a statistical analysis. This Peto analysis indicated [a] statistically significant pair-wise increase in carcinomas only at the 500 ppm level. No increases in carcinomas were noted at the higher doses nor at any doses for the combined adenomas and carcinoma analysis.

In summary, I see no reason to change our analysis of the tumor data based on Dr. Dementi's October 28, 1999, comments and I believe that the CARC has been consistent in its rationale and analysis of the presence of tumors at excessive and at adequate doses."

The follow-up memorandum from Dr. Dementi to Dr. Burnam (ref. 18) does not provide additional factual arguments. I feel that the guidelines cited above provide for the use of scientific judgement regarding the use of tumor data at excessive doses. In the case of the malathion rat study, the CARC determined that tumor responses occurring only at excessively toxic doses were not appropriate for use in dose-response extrapolation. The data were however, considered to be supporting evidence of carcinogenicity—**and were not discarded**.

12) Application of general principles of competing toxicity and increased mortality in mitigating expression at excessive doses of a tumorigenic dose-response occurring at acceptable lower doses (no specific references given, used ref. 4, 7, 17)

Dementi Summary: "In my opinion, having cited authoritative sources, competing toxicity and increased mortality at excessive doses may diminish or even preclude tumorigenic responses identified at lower doses. Furthermore, in consideration of the potential for such compromises of tumor expression to occur at excessive doses, negative or diminished findings at such doses cannot be accepted as negative evidence of carcinogenicity. It is more acceptable, as I understand, to accept positive findings at excessive doses unless (according to EPA's draft Cancer Guidelines) it can be shown such tumorigenic responses resulted from toxicity as opposed to tumorigenicity of the test material. I am not satisfied CARC has made proper use of these concepts, specifically, in discounting certain tumorgenic findings at dose levels considered excessive without demonstrating these arose secondary to toxicity, while on the other hand accepting diminished tumorigenic responses at excessive doses as negative evidence. There have been no statements from CARC clarifying its philosophy.

"I must add that in the case of liver tumorigenic responses in female rats in the combined chronic toxicity/carcinogenicity study with malathion, I concur with CARC's interpretation at all doses, including that for the highest dose group, as being consistent with my understand of the principles at issue here, i. e., there is no evidence the tumorigenic response observed at the highest dose, the only dose level considered excessive in females, was due to anything other than the tumorigenicity of the test material."

Response:

While the CARC does acknowledge that competing toxicity may impact the expression of tumors, it is difficult to determine this as a cause without appropriate supporting and /or mechanistic data. The cancer guidelines (ref. 25) note that:

> "…(c) If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity."

I feel that this should apply to specific tumor types as well. The CARC can not make suppositions about the validity of a negative (or the lack of a) tumor response at excessive doses without solid scientific evidence. In most cases however, the CARC considers their decisions to be protective since any tumors that might be masked by competing toxicity are usually at excessively toxic doses. Therefore, I feel no additional evaluation of competing toxicity is warranted.

13) Acceptability of the rat combined chronic toxicity/carcinogenicity study to evaluate carcinogenic potential in male rats (no specific reference given, used ref. 8, 10, 17, 18)

Dementi Summary: "I have difficulty accepting CARC's decision concerning acceptability of the study as essentially a *negative* study in male rats, specifically the male F344 rat. Discounting the top two doses as excessive, and accepting the lower dose levels, in my opinion precludes testing at adequately high dose levels. The findings suggest the need for another dose group somewhere between the 500 and 6000 ppm dose groups. It may be the F344 rat is a poor model due to competing toxicity. On the other hand, if CARC accepted the study as demonstrating tumorigenic findings in males at the lower doses, perhaps that would be the end of it. Given the male rat assessment is thus confounded, greater reliance must be placed on findings in females, i.e., as carrying more weight than a single gender finding."

Response: (also see response to item 11)

The 6000 and 12,000 ppm dietary concentrations were both considered excessive for male rats based on mortality [total mortality 18/55(33%)**, 14/55(25%), 26/55(47%), 39/53(74%)**, 56/56(100%)** for controls to the high dose] and cholinesterase inhibition in all three compartments. In contrast, the 500 ppm dose group was considered adequate to evaluate carcinogenicity. Although not explicitly stated in the CARC Report, there was evidence of some toxicity: 1) a non-statistically, but probably biologically significant increase in mortality at this concentration; and 2) a decrease in plasma cholinesterase (29%, $p \leq 0.01$). **I recommend that this be included in the new CARC Report**. Therefore, it is considered to be appropriate to use the 500 ppm dose when evaluating this study for the carcinogenic potential of malathion in the male rat—without requiring any intermediate doses. In addition, for the reasons noted above, the CARC felt that requiring a new test with the male rats was not necessary–any additional information would not alter the cancer assessment and classification which is already, "likely human carcinogen." I feel that the data would have supported the 500 ppm dose as adequate in the hypothetical situation where it was the high dose in the study.

14) Adequacy of Q* method to address risks posed by low dose tumorigenic findings, e.g., liver tumors in the female rat at 100/50 ppm, in the absence of a NOEL (ref. 15, 16, 17)

Dementi Summary: "This is a philosophical question raised by me that has not been discussed at any of the CARC meetings as I recall. It is my concern that to the extent low dose tumorigenic findings occur at more elevated incidences than expected based on those incidences at much higher doses (e.g. the female rat liver tumor response), for whatever reason, such as a change of mechanism across the dose range, can the Q* calculation, employing all doses, be expected to address risks posed at the low dose level. I am concerned low dose findings in this assessment, close to those that minimally inhibit cholinesterase are of peculiar concern to the public health, and petition for additional expert comment on the utility of the Q* method to deal with this. This is more a gut feeling than one borne of any particular expertise or evidence I bring to the table. The Q* method has been used by CARC to address public health risks based on the female liver tumorigenic response."

Response (see last paragraph, page 2):

In my opinion, the CARC meetings and documents are not the appropriate forum for philosophical discussions. These are better deliberated in Agency workgroups. The above philosophical question is generic and does not apply specifically to malathion. Therefore, my response is general and does not address malathion specifically. The CARC is required to follow the EPA Cancer Guidelines. The 1986 guidelines and 1996 and 1998 drafts are fairly specific regarding when linear extrapolation is to be deviated from. In order to use some other form of modeling, there has to be support from mechanistic data. The 1986 guidelines use the Q_1* (the linearized multistage procedure), while the new draft guidelines (1996 and 1999) discuss extrapolation from an LED_{10} or ED_{10}. Due to the fact these are still draft, and the method of quantitative risk assessment is still undergoing modification and clarification, HED has continued to use the Q_1* when linear extrapolations are warranted. Both the Q_1* and LED_{10} or ED_{10} methods are similar types of low dose linear extrapolation models which use all of the data at the high and low doses.

Reference 21 - Items identified by Dr. Dementi as either incorrect or inconsistent.

1) Dr. Dementi's comments concerning CARC Report p. vi, paragraph 3:
(see last paragraph, page 2 of this memorandum)

The final CARC Report already was corrected to say "two tumors per dose level," therefore no follow-up action is needed.

In the same paragraph, Dr. Dementi expressed concern about the use of the expression "mainly adenomas" when referring to the liver tumor increase in females.

I feel that this concern may be valid since there are as many carcinomas at each dose as adenomas, with the exception of the 6000 ppm dose. This paragraph will be modified to say, "There was no statistical significance for carcinomas." In addition, the expression, "(an adenoma and carcinoma)" will be added to the comment that there were two tumors at each of the two low doses.

2) Dr Dementi identified an error in the date given for the Original Pathology Report in the table 1 title. This report was completed in 1994 while the table listed the date as 1997—the date that the statistics in the table were completed.

The table title will be modified to read: "Based on the Original 1994 Pathology Report"

3) Dr. Dementi expressed concern that there are inconsistencies between the methods for evaluating male rat follicular cell and c-cell tumors of the thyroid.

These two tumor types are discussed in the *CARC Responses to Issues Raised By Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion,* items 2 and 3 above.

4) Dr. Dementi noted that there appeared to be a discrepancy between the way the nasal and oral rat tumors were evaluated.

I discussed this concern in items 6 and 7 above, where I recommended that the oral tumor response be reevaluated by the CARC for the reasons that Dr. Dementi enumerated.

REFERENCES

from Brian Dementi:

1 Dementi, B.(1997). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 26, 1997.**

2 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **February 23, 1998.**

3 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 9, 1998.**

4 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jerry Hardesty, dated **May 4, 1998.**

5 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 29, 1998.**

6 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Sanju Diwan, Executive Secretary, Cancer Assessment Review Committee, dated **February 11, 1999.**

7 Dementi, B.(1999). *Recommendation to CARC Members* from Brian Dementi, Toxicology Branch 1, dated **February 24, 1999.**

8 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **April 1, 1999.**

9 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 27, 1999.**

10 Dementi, B.(1999). Addendum to Malathion Qualitative Risk Assessment Based on Fischer 344 Rat Dietary Study. *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 18, 1999.**

11 Dementi, B.(1999). Malathion Combined Chronic Toxicity/Carcinogenicity Study in the F344 Rat (MRID No. 43942901). *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 7, 1999.**

12 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 21, 1999.**

13 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 13, 1999.**

14 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 22, 1999**.

15 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **September 21, 1999.**

16 Dementi, B.(1999). *Memorandum:* Comments on September 20, 199 Draft CARC Report on Malathion. Brain Dementi, Toxicology Branch 1 to Jess. Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 6, 1999.**

17 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 28, 1999.**

18 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 12, 1999**.

19 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **December 7, 1999.**

20 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **January 12, 2000.**

21 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 7, 2000**.

22 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 9, 2000.**

Others:

23 - Burnam, W.(1999). *Memorandum:*"Tumors at Excessive Doses," William Burnam, Science Analysis Branch to members of the current CARC, dated **November 5, 1999.**

24 Caldwell, D.J. (1999). Review of Mononuclear Cell Leukemia in F-344 Rat Bioassays and Its Significance to Human Cancer Risk: A Case Study Using Alkyl Phthalates. *Reg. Tox. And Pharm.* 30:45-53.

25 EPA Guidelines for Carcinogen Risk Assessment, preliminary drafts, 1996, 1999.

26 The FIFRA Scientific Advisory Panel report, "A Set of Scientific Issues Being Considered by the a Agency in Connection with DDVP (Dichlorvos) Risk Issues," meeting date July 30, 1998.

ATTACHMENT 26

Date: 27-April-2000

MEMORANDUM

SUBJECT: Responses to Concerns Expressed by Dr. Brian Dementi (memo dated 9-Feb-2000) Regarding the Final HED Cancer Assessment Review Committee Report (2-February-2000) for Malathion

PC Code: 057701
DP Barcode: D264571
Submission #: S529758

FROM: Marion Copley, D.V.M., D.A.B.T.
CARC Member
Registration Action Branch 1
Health Effects Division (7509C)

TO: William Burnam, Committee Chair
Carcinogen Assessment Review Committee
Science Analysis Branch
Health Effects Division (7509C)

Margaret Stasikowski, Director
Health Effects Division (7509C)

As you requested, I have reviewed the remainder of the comments submitted by Dr. Brian Dementi (memorandum titled: Comments on February 2, 2000 CARC report on malathion, dated: 9-February-2000, to Jess Rowland: Executive Secretary, Cancer Assessment Review Committee. This memorandum identifies items in the 2-February-2000 CARC Report (called CARC Report in this memorandum) that Dr. Dementi considered to be either factually incorrect or unclear. All comments expressed in Dr. Dementi's other 21 documents have previously been addressed (memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion, dated 30-March-2000, from Marion Copley, to William Burnam and Margaret Stasikowski).

This memorandum only responds those concerns not addressed in the above cited memorandum. It presents my interpretation of the CARC position regarding the comments raised by Dr. Dementi and does not in itself revise the CARC position as stated in its report. Errors or areas needing clarification will be corrected in the CARC # 2 document resulting from the April 6, 2000 CARC meeting. As it the usual procedure, the draft CARC # 2 document will be circulated to all committee members for comment prior to finalization.

It should be noted that my references to female rat liver tumors are based on the data as it existed as of the 2-February-2000 CARC Report. Cheminova has recently submitted revised tumor incidences for these tumors based on a PWG evaluation. The results of the new submission will not be addressed in this memorandum. Therefore, several of my comments regarding female rat liver tumors may not apply if the new values are accepted.

Dr. Dementi's comments refer to a draft rather than the final 2-February-2000 CARC Report. Therefore, the page and paragraph references are not the same as those in the final CARC Report.

1. **Comment regarding viii - 3d paragraph** - Dr. Dementi requested that the brain cholinesterase values for 8000 and 12,000 ppm in the third paragraph of the executive summary be listed separately since he doesn't feel that the 20 % inhibition at 8000 ppm is excessive.

   I don't feel that this change adds anything to the document's clarity. This information is in the body of the report. In addition, as described in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion in Item 15, the determination of excessive toxicity is not based solely on the brain cholinesterase inhibition (30-March-2000).

2. **Comment regarding page ix - 1st paragraph:** The Committee further concluded that there is evidence of carcinogenicity in female rats (but not males) which manifested as liver tumors at all dose levels and tumors of the nasal mucosa at 6000 ppm, although nasal tumors were also seen at 12000 ppm (a dose considered excessive). *Nasal tumors were also observed in males, one each, @ 6000 ppm and 12000 ppm.* ***In reference to my October 6 comments on your September 20, 1999 draft, you say ok for noting the male nasal tumor finding,*** *but didn't make the change.*

   I had already observed this inconsistency. However, based on the 12-April-2000 meeting, this entire section will be modified.

3. **Comment regarding page ix - 3rd paragraph**: ....was slightly outside the historical control range and well above the mean value in a small historical control data base. *There is no large historical data base that is appropriate, since this was an 18-month study, while the NTP data base is for 2-year studies. The performing laboratory data base is very inadequate.*

   I don't feel that this change would add anything to the document's clarity.

4. **Comment regarding page 2nd paragraph:** ....and marked brain (20 to 43%) cholinesterase inhibition. *As stated on p. viii, the 20-43% should not be represented for both 8000 and 16000 ppm, as 20% inhibition at 8000 ppm may not be viewed as excessive by many people. Your case is not as strong for 8000 ppm as for 16000 ppm, though I believe, as stated elsewhere, both are inadequate arguments to discount these doses.*

   I don't feel that this change would add anything to the document's clarity.

4. **Comment regarding page x - 4th paragraph:** ....and that the liver tumor incidences at 6000 and 12000 ppm (although considered to be excessive doses).... *Should be revised*

to say: (although 12000 ppm is considered to be an excessive dose)

I had already observed this inconsistency and Dr. Dementi is correct. It will be corrected in the CARC document resulting from the 12-April-2000 CARC meeting.

6. **Comment regarding page x - 6th paragraph:** Thus, the relevant incidence for the tumor type in question is 2/4000 control males. *In the same NTP data base, the reported incidence for neoplasms of the nasoturbinate tissues was zero in approximately 4000 control female F344 rats. (Jess, see p. 62 of the study DER for confirmation of this number)*

   This was already included in the final CARC Report.

7. **Comment regarding page xi - 1st paragraph:** However, the Committee concluded that a systemic effect could not unequivocally ruled out. *A local effect, as opposed to a systemic effect is less likely for the tumors in females which were identified in section 5, the most remote nasal region and where little other histopathology was seen. In my recall, the discussion was such that no evidence exists to conclude whether via inhalational or systemic route or both.*

   I am recommending that the following be added to the CARC document resulting from the 12-April-2000 CARC meeting: **"However, there was no evidence to support or refute that the toxicity was due to exposure by the inhalation or systemic route."**

8. **Comment regarding page xi 3rd paragraph:** ....because (1)....; (2)....; (3)....; (4)....; and/or (5).... *See discussion for each tumor type for rationale as not treatment-related. This lumping together of reasons confuses the reader as to reasons employed for each tumor type. The statements should be specific for each tumor, or referred to the text as I have suggested.*

   The rationale for each tumor type has been separated out on the report for the 12-April-2000 CARC # 2 meeting.

9. **Comment regarding page xi last paragraph:** Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats when tested at doses that were judged to be adequate to assess carcinogenic potential. *Its fine to say CARC voted malaoxon was not carcinogenic, but you should acknowledge the positive trend, as well as the findings in the high dose group as being statistically significant for leukemia, and say why this finding was discounted: reason - say dosing was excessive, according to CARC.*

   I am recommending that the following be added since this is consistent with what was done for several of the tumors that were determined to be unrelated to malathion in the rat: "**MCL was not considered to be treatment related since statistical significance was seen only in males at a dose that was determined to be an excessive dose, there was no dose-response, and the incidences were within the historical control range of the testing laboratory**." I am also recommending that the words "the increase" in the original sentence from the body of the report be changed to "**statistical significance**" since that is actually what the tumor table indicates.

10. **Comment regarding page xii 3rd paragraph:** The Committee further observed that it

is plausible that tumor occurrences in these studies are dose-limited (i.e., tumors are induced only at excessive doses), however, mode of action studies to demonstrate this hypothesis are not available. *This would not be consistent with the findings of rare liver tumors in females at both low dose levels**, without qualifying the reality of the biological significance of tumors in both lower dose groups.***

Based on the outcome of the 12-April-2000 CARC meeting, this entire section will have to be modified.

11. **Comment regarding page 2 1st full paragraph:** ....was no clear evidence of carcinogenicity due to malathion or malaoxon administration in most *(actually three)* of the studies *(under review by NTP). This statement needs to be revised to reflect NTP's conclusions. Thyroid C-cell tumors was a positive finding statistically (trend and high dose pair-wise)(both sexes) in the malaoxon study.*

    I don't feel that this change adds anything to the document's clarity.

12. **Comment regarding page 3 4th paragraph:** There was also a significant.....for combined adenomas/carcinomas ($p<0.01$). *Though not statistically significant, the increase at 800 ppm was over 4-fold that of the control, and thus contributes to the remarkably positive trend ($p = 0.000$).*

    I don't feel that this change adds anything to the document's clarity. This is obvious in table 1 of the report and does not need to be added to the text.

13. **Comment regarding page3 5th paragraph:** ....doses but not at the mid dose (800 ppm) *(though increased over 4-fold that of the control, 9% versus 2%; why do you not wish to acknowledge this simple truth?*

    I don't feel that this change adds anything to the document's clarity. This is obvious in table 1 of the report and does not need to be added to the text.

14. **Comment regarding page4 2nd line:** As reported in the original study report of 1997. *Study date: October 1994; date of DER: February 1995; **hence, 1997 is incorrect)***

    This error will be corrected in the CARC document resulting from the 12-April-2000 CARC meeting. It was already addressed in my previous memorandum (30-March-2000).

15. **Comment regarding page4 footnote e:** Two males at 100 ppm had both an adenoma and a carcinoma*; a third and possibly a fourth male had two carcinomas of the liver; i.e. in this dose group there are three and possibly a fourth mouse exhibiting liver tumor multiplicity.*

    The purpose of this footnote is to explain why the combined totals are greater than the sum of the adenomas and carcinomas. The issue of multiplicity is presented in table 3b. No change is needed.

16. **Comment regarding page5 footnote c:** *Same revision need here as in footnote e on p. 4 given above.*

The purpose of this footnote is to explain why the combined totals are greater than the sum of the adenomas and carcinomas. The issue of multiplicity is presented in table 3b. No change is needed.

17. **Comment regarding page 5 last paragraph:** Increased incidences of adenomas, carcinomas and combined adenomas/carcinomas were seen at 100 ppm and 800 ppm, but none of the increases showed either statistical significance or a dose-response relationship. *To the contrary, the 800 ppm group in my view evidences a clear dose trend with respect to the 8000 and 16000 ppm groups, i.e. control (7%), 800 ppm (16%), 8000 ppm (27%) and 16000 ppm (96%). The increase at 100 ppm (19 %) is anomalous and suggestive of a different mechanism, involving more carcinoma.*

    This comment has already been addressed in memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item 1 (30-March-2000).

18. **Comment regarding page 6 last paragraph:** Dr. Brennenke, the consulting pathologist, commented that in the evaluation of carcinogenicity, "tumor bearing animal" counts as one *(statistically or numerically)* regardless of the number or multiplicity of any tumor type. *In comparing a control versus a dose group, it is well recognized that multiplicity is a weighing factor in tumorigenesis assessment, particularly in that multiplicity is evidence of earlier onset or increased rate of progression or development of the disease. Please see for example OSTP (1985). I do not understand why the committee does not wish to acknowledge the significance of multiplicity.* Although carcinomas were observed.....the incidences showed neither a dose-response relationship nor statistical significance at any dose level. *Carcinomas are rare (few) in the historical controls, yet many appear in this study.* In addition, tumor incidences at the two high doses should be considered carefully since these dose levels were determined to be excessive for assessing carcinogenicity..... *To be fair with your reasoning, to the extent you seek to downgrade the importance of findings at 8000 and 16000 ppm, you should acknowledge certain of the concerns that the findings at 100 ppm may be real, namely: 1) multiplicity; 2) carcinomas; 3) nearly positive pairwise (p = 0.075); 4) numerically increased incidence, nearly 3-fold; 5) possibly different mechanism at low dose; 6) female rat also exhibited liver tumor response, "cannot be discounted", in the same low dose range of 100/50 ppm, for hepatocellular adenoma and carcinoma; 7) profoundly positive dose trend in mice, p = 0.000. I stand amazed over the imbalance in your treatment in so positive a study.*

    I was going to recommend that the following be added to the CARC document resulting from the 12-April-2000 CARC meeting: "**and observed an increase in multiple adenomas only at the high dose. The significance of this is unclear since this is an excessively toxic dose.**" However, based on the 12-April-2000 CARC meeting this section will have to be modified.

19. **Comment regarding page 7 1st paragraph:** In the 5 historical control studies, the incidences of liver carcinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in another study (6.4%). *In reference to my 10/6/99 comments on this point, you say "This is a comment". The point nonetheless is, what does the committee say, or what guidelines may there be that address the usefulness of a control data base, particularly when so small in number of studies and animals tested. Surely some comment here is merited. When a control data base is so weak, greater reliance must*

be placed on the contemporanous control.

There were 5 studies in the historical control data base of the testing facility. Although this is not a large number, it represents about 250 animals. This is not "weak" as described above. in making their decision about the biological relevance of the liver tumors in mice, the committee considered many factors, not just the historical control data. The issue of mouse liver tumors was also addressed in memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item 2 (30-March-2000).

20. **Comment regarding page 7 2nd paragraph:** Also in the NCI study, among females, the combined adenomas/carcinomas incidences were *2% at 0 ppm*, 0% at 8000 ppm and 4% at 16000 ppm in contrast to the present study where the tumor incidences in females were *2% at 0 ppm*, 19% at 8000 ppm and 84% at 16000 ppm. The committee noted that the tumor responses in the present study at the same dose levels was more pronounced than those seen in the NCI study. *Suggested preferred sentence: The committee noted as inexplicable among females the absence of an hepatocellular tumorigenic response in the NCI study, versus the clear positive findings for females in the new study.*

    I recommend that the control values be added as noted in this comment. In addition, while I didn't use the exact wording from the comment, I am recommending that the last sentence be modified.

21. **Comment regarding page 9 table 5 footnote:** Incidences presented are the <u>total of the lesions</u> observed in the 5 sections of the nasal tissue. *I can see the advantage of totalling the findings from all 5 sections, however a disadvantage to this approach is that it does not reveal that section 5 had little pathology, yet it was in section 5 that both nasal tumors in females were found. This suggests the tumors were de novo and not secondary to other pathology, which is very important. You should acknowledge that little of these findings were in section 5.*

    Tumors in the rat are in histologic slides with section five. Table 5 above refers to the mouse lesions. The rat data is presented in table 17 in the 2-February-2000 CARC report (now presented in table 18). there is already a statement in the CARC report stating that most of the non-neoplastic lesions did not occur in section 5, the section where both nasal tumors in the females occurred.

22. **Comment regarding page 9 last paragraph:** At necropsy, liver "masses" were seen at all dose levels*, but not in the control.*

    The following will be added to the CARC report. "At necropsy, liver "masses" were increased over controls in all male dose groups and at 16,000 ppm in females."

23. **Comment regarding page 10 1st paragraph:** The Committee further noted that the 8000 ppm.....and the 16,000 ppm.....dose was more than twice the Limit Dose. *You've still disregarded my note that these doses are not all that high, given the Guideline 5% of the diet cut off, now that the study is done.*

    I don't feel that this change adds anything to the document's clarity.

24. Comment regarding page11 3rd paragraph: There were no statistically significant increases in hepatocellular tumors at any dose level in male rats. *Explain why males may be negative - dosing (mortality) too great for male assessment.*

The report will be modified to explain why the males are considered negative for liver tumors.

25. Comment regarding page11 4th paragraph: In addition, the incidences of these two tumor types *in the 100/50 and 500 ppm groups also* exceeded the historical control.

This sentence will be modified in the CARC document resulting from the 12-April-2000 CARC meeting.

26. Comment regarding page11 last paragraph: The Committee also concluded that the liver tumor incidences at 6000 ppm and at 12,000 ppm (although *12000 ppm was* considered to be *an* excessive doses *(dose))* provide positive evidence of carcinogenicity. *There was no NOEL for hepatocellular tumorigenicity in this study.*

We usually do not use the term NOEL with regards to cancer. This entire section will be changed due to the results of the 12-April-2000 CARC meeting.

27. Comment regarding page12 1st paragraph: This conclusion was based on: 1)..... ***add*** *5) carcinomas also exceed; 6) extremely rare in females per NTP data base.*

The note that carcinomas also exceed the historical control data base will be added. The note about the NTP data base however, was already implied earlier when the values were given as 0 out of 901.

28. Comment regarding page12 table 7: *As presented in the table, p values of $\leq$ 0.05 are in bold type. Values so close to p = 0.05, e.g. p = 0.063 and 0.085 as shown in the table merit some emphasis, such as grey highlight. An exceedingly rare tumor such as carcinoma of the liver of the female F344 rat need not be statistically significant at p = 0.05 to be real, but in this case the p values being very close to the 0.05 criterion should be considered real as supported statistically for such a rare tumor type.*

I don't feel that this change adds anything to the document's clarity. Statistical significance needs to be taken together with all other information provided by the study.

29. Comment regarding page 12 last paragraph: This was a nasal tissue reevaluation, and oral tissue findings (tumors of the palate and *alveolus of the* tooth) were.....

This change will be made to the CARC document resulting from the 12-April-2000 CARC meeting.

30. Comment regarding page 13 1st paragraph: Therefore, the relevant incidence for the tumor type in question is 2/4000 control males. *As noted on your p. x, the NTP data base incidences of nasoturbinal tumors is zero in approximately 4000 control F344 female rats.*

The executive summary in the final CARC Report was already modified so that this inconsistency does not exist.

31. Comment regarding page 14 3rd paragraph: The Committee postulated that direct contact with malathion (by volatilization from the feed *(no)* or by inhalation of the feed through the nose (*possibly yes))* was a plausible explanation for the nasal tumors; however, it was concluded that a systemic effect could not be unequivocally ruled out., *particularly since the nasal tumors in females were in the back of nose (section 5) where little other evidence of a local effect was observed. I prefer and believe more accurate is your paragraph in the September 20 draft (p. 11). I believe the committee was persuaded that malathion volatility is too low to be a significant factor here.* The Committee noted .....is required based on the *lack of NOAELs for cholinesterase inhibition and non-neoplastic lesions of nasal tissues in both the 2-week range-finding study (MRID 44554301) and the 90-day study (MRID 43266601) (HIARC report). The fact that nasal histopathology was observed after only 2-weeks of treatment would not have been identified in Guideline subchronic studies and is a matter of concern with respect to effects by inhalation.*

I am recommending that the following be added to the CARC document resulting from the 12-April-2000 CARC meeting: **"However, there was no evidence to support or refute that the toxicity was due to exposure by the inhalation or systemic route."**

32. Comment regarding page 14 1st paragraph: Palate tumors were observed..... These tumors were not attributed to malathion treatment due to lack of statistical significance *(statistical significance is not required for rare tumors)*, and absence of a dose-response in either sex. *A papilloma at 6000 ppm and a carcinoma at 12000 ppm is some evidence of a dose related response. There was no such trend for the nasal tumors**, and there were malignant tumors among those of the oral cavity**. You should remind the committee, the original reason for discounting the oral tumors versus the nasal tumors was the alleged lack of rarity of the former. However, I explained in memoranda that the squamous cell tumors of the oral palate are as rare as nasal tumors in NTP's data base. So now other reasons, also incorrect, are cited. You should say that squamous cell tumors of the palate, like nasal tumors, are extremely rare in the NTP data base. Incidence appears to be zero in both sexes, or possibly but one such tumor exists in the entire NTP data base as explained in my memoranda to the chairman.*

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 7 (30-March-2000). In addition, this issue of nasal tumors was readdressed by the CARC on 12-April-2000. The new CARC document will reflect the revised CARC conclusions.

33. Comment regarding page14 last paragraph: The Committee concluded that the thyroid follicular cell tumors are NOT treatment-related since there is neither a pair-wise significance nor a dose-response relationship for any tumor type.....; only a trend was seen for the combined tumors. *A trend is a dose-response relationship finding. Elevated mortality and competing toxicity at 6000 and 12000 ppm in males may have compromised <u>full</u> expression of these tumors at 6000 and 12000 ppm. Peak incidence arguably occurred somewhere between 500 and 6000 ppm, at a dose not tested. Jess, I find it very disturbing that in the earlier draft it was claimed that the conclusion was based on the fact that when the two excessive toxic doses (6000 and 12000 ppm) are excluded, there are no increases in tumors. Yet, when I noted that the same argument fails in the case of C-cell tumors, you delete the argument here. If the concept is*

important, as the committee has recognized it to be, you should adhere to it in both thyroid tumor cases. Also, did the full committee revise its vote?

The issue of thyroid follicular cell tumors has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 3 (30-March-2000).

34. Comment regarding page 16 2nd paragraph: The Committee also observed that when the top two doses (6000 ppm and 12,000 ppm) were excluded (Table 10b) from the analysis*, as appropriate, particularly since these two dose groups have been discounted by the committee due to excessive dosing,* there was a dose-related increase (2%, 4% and 13% at 0 ppm, 50 ppm and 500 ppm, respectively)*, yielding a remarkably positive trend, p = 0.006,* a pair-wise significance (p = 0.013) at 500 ppm, and the increases at both doses exceeded the mean historical control incidence (6/239; 2.5%) for carcinomas in male rats. However, the pathologist *(what pathologist, i.e. who said this and on what occasion? I recall Swenberg saying pituitary adenomas and carcinomas difficult,* ***but do not recall it being claimed by a pathologist at CARC meetings, and this is the first draft of CARC meetings in which this claim is recorded*****)** stated that thyroid C-cell adenomas and carcinomas are difficult to differentiate. *The diagnoses have been rendered and not refuted by any peer review. Furthermore, there likely is a significant conversion of adenoma to carcinoma at 500 ppm, per discussion in my May 18, 1999 memorandum to Burnam.....*However, the Committee noted that although excessive mortality was also seen in females at the top dose (64% at 12,000 ppm) liver tumors were seen at this dose. *This is not to say incidence would not have been greater yet for liver had survival been longer, i.e. the finding was seen, but still may have been compromised. Also, consider leukemia (males) in this study for effects of 12000 ppm in mitigating expression.*

The issue of thyroid C-cell tumors has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item #2 (30-March-2000). The reference to the pathologist will be deleted from the CARC document resulting from the 12-April-2000 meeting.

35. Comment regarding page 16 3rd paragraph: The combined tumors were determined to be the most appropriate tumor type for evaluation due to the difficulty in distinguishing the individual tumor types (i.e., adenomas and carcinomas). *I disagree with this assertion. Can you show me where in the record of any meeting this was voted on the basis of this principle,* ***furthermore diagnoses have not been revised by any independent examination of the slides by a pathologist.*** *Thyroid C-cell tumors were of principle concern as a finding in the earlier NCI studies, particularly for the malaoxon F344 rat study. See your own list on p. 2 of this document. On this tumor type I stick with my comment on the earlier draft, p. 5 of my 10/6/99 comments. Also, you still do not acknowledge C-cell tumor multiplicity for the 500 ppm group in your reasoning. Furthermore, you say nothing about my May 18, 1999 memorandum to the chairman on C-cell tumors. In summary. Your comments on C-cell tumors are not balanced, presenting both sides of the question.*

The issue of thyroid C-cell tumors has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item #2 (30-March-2000).

36. Comment regarding page 20 1st paragraph: The Committee concluded that the testicular tumors are NOT treatment related. *Why did we perform the Peto test on this data if we were not prepared to honor the findings? The Peto test takes mortality into consideration. If conducted properly, as we assume it was in this case, the only explanation is that the tumor type, in mass (i.e. close to 100% incidence) in all groups was unexpected among animals of such high mortality. You do not need serial sectioning to perform the Peto test.* ***Furthermore, the burden of proof otherwise rests with those who denounce the findings for some hypothetical reason.***

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 5 (30-March-2000).

37. Comment regarding page 21 1st paragraph: The Committee concluded that mononuclear cell tumors in male and female rats are NOT treatment related based on the lack of statistical significance at any dose level. *Please note and say that there was statistical significance for the increased female leukemia incidence at 100/50 ppm* ***(p = 0.025)****. Furthermore, while not quite significant by the p = 0.05 criterion, the increase at 500 ppm was significant at p = 0.059. This is very important in consideration of all of the data, males included, which I discussed in my April 27, 1999 memorandum to the chairman. Namely, that mortality due to leukemia among leukemia bearing animals was increased in a dosing-related manner, constituting evidence of increased progression under the OSTP (1985) definition of carcinogen. Also, leukemia was a finding at 2000 ppm in the recent malaoxon study and in the malathion NCI study (see p. 2 of your paper). All of these facts should be presented here to provide your audience a more balanced assessment.*

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 4 (30-March-2000).

38. Comment number 2 about page 21 1st paragraph: .....and the 1996 malaoxon *(no!)* studies.

The CARC has determined that MCL was "not treatment related [in the malaoxon rat study] since statistical significance was seen only in males at a dose that was determined to be an excessive dose, there was no dose-response, and the incidences were within the historical control range (15-36%) of the testing laboratory.

39. Comment regarding page 21 2nd topic: C. Non-Neoplastic Lesions. *The whole question of nasal tissue vulnerability to malathion is one which has not been adequately addressed by HIARC or CARC. While another inhalation study has been required that hopefully will shed more light on the subject, given our present knowledge, what can be said at this time concerning risk? It is uncertain whether the nasal histopathology in the rat (and mouse also) in the chronic feeding studies was a local effect or a systemic effect. I am inclined to believe both. In any case, the chronic feeding studies and the subchronic and dose range-finding inhalation studies all attest to a remarkable sensitivity of nasal tissues to malathion that has not been adequately addressed, particularly for inhalation exposures, the effect of which on nasal tissue would be exacerbated by oral ingestion. CARC has provided a Q* for nasal tumors in an oral feeding study, but does this address risk for persons exposed chronically by the*

inhalational route? This report should say something about this concern.

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 6 (30-March-2000).

40. Comment regarding page21 3rd paragraph: Mortality was increased in males at 6000 ppm and in both sexes at 12000 ppm..... *The increase in mortality among males appears to extend down to 500 ppm, 47% mortality versus 33% in the control.*

This will be modified for completeness in the CARC document resulting from the April 6, 2000 CARC meeting.

41. Comment regarding page24 table 20 [should be 21]: Mononuclear Cell Leukemia in Rats Fed Malaoxon for 24 Months. *Add to table legend: Method of Peto, et al (1980) per September 22, 1997 letter of Huntingdon Life Sciences to Dr. Judy Hauswirth*

This will be modified for completeness in the CARC document resulting from the 12-April-2000 CARC meeting..

42. Comment regarding page 24 4th paragraph: The Committee concluded that mononuclear cell leukemia.....and F344 (1979, NCI-malaoxon) studies, *but was of concern in the NCI malathion F344 rat study (males) - see p. 2 of your report. Again, balance is important, both pro and con.*

The early part of the CARC document referred to above stated, "The CPRC agreed with the NTP re-analysis that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration…However, the committee felt that there were many issues regarding the adequacy of each study which needed to be addressed before a firm conclusion regarding the carcinogenic potential of malathion could be made." The new studies do not provide support that the tumors observed in the early studies are treatment related. Therefore, adding anything in section referred to above (about page 24) would be misleading.

43. Comment regarding page 25 1st paragraph: *Your numbers are now <u>good</u> and show a more remarkable effect at 2000 ppm than at 1000 ppm, particularly brain cholinesterase inhibition. It is not clear to me what criteria CARC follows in saying cholinesterase inhibition is excessive or not. Arguably, the blood enzyme inhibitions at 1000 ppm are excessive. For cancer assessment, though, I really don't think so, absent clinical signs that are unacceptable.*

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 9 (30-March-2000).

44. Comment regarding page28 2nd paragraph: In a subchronic inhalation study,.....was 2.01 mg/L (MRID 43266601). *HIARC is requiring another inhalation study to address absence of NOAELs and to further characterize nasal histopathologic responses.*

This thought was already included in the final CARC Report.

45. Comment regarding page30 3rd paragraph: When compared with historical control ranges.....historical control range (0 to 6.4%). *You should show the mean as you did on p. 31, 2nd paragraph for the rat.* No carcinomas were seen at 16,000 ppm while the incidence of carcinomas at 100 ppm (7%) was slightly outside the historical control range*, and well above the mean value in a small historical data base of the performing laboratory. Unfortunately, NTP's data base is for full 2-year studies and cannot be used in this comparison.*

The following has been added to the text to clarify this: "data for mean incidences are not available."

46. Comment regarding page31 3rd paragraph: However, this conclusion was lessened since these tumors occurred in mice only at doses which caused severe plasma (90 to 95%) and red blood cell (92 to 96%) cholinesterase inhibition and marked brain (20 to 43%) cholinesterase inhibition. *Again, you should break out brain cholinesterase inhibition for 8000 and 16000 ppm. People may not be convinced that 20% inhibition at 8000 ppm is excessive such as to support discounting 8000 ppm as an excessive dose.*

I don't feel that this change adds anything to the document's clarity. In addition, as described in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion in Item 15, the determination of excessive toxicity is not based solely on the brain cholinesterase inhibition (30-March-2000).

47. Comment regarding page31 4th paragraph: However, this conclusion was lessened since these tumors occurred in mice only at doses which caused severe plasma (90 to 95%) and red blood cell (92 to 96%) cholinesterase inhibition and marked brain (20 to 43%) cholinesterase inhibition. *Again, you should break out brain cholinesterase inhibition for 8000 and 16000 ppm. People may not be convinced that 20% inhibition at 8000 ppm is excessive such as to support discounting 8000 ppm as an excessive dose.*

This has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 8 (30-March-2000).

48. Comment regarding page32 2nd paragraph: Therefore, the relevant incidence for the tumor type (adenomas) in question is 2/4000 control males. *In the same NTP data base, the reported incidence for neoplasms of the nasoturbinal tissues was zero in approximately 4000 control female F344 rats.*

See comment 30 above.

49. Comment regarding page32 3rd paragraph: The Committee postulated that direct contact with malathion (by volatilization from the feed..... *See p. 13 for my comments on the volatilization issue. I question the committee accepted the idea.*

See comment 31 above.

50. Comment regarding page32 last paragraph: C. <u>Other Tumors</u> *See my comments on p. xi in opposition to this language. You have glossed over the oral cavity squamous cell tumors, which are as rare as the nasal tumors. I remain concerned over the finding*

of eight exceedingly rare tumors in dosed groups only in the nasal tissue histopathology re-evaluation, ***that did not include proper or complete histopathology assessment of the oral cavity.***

Part of this has already been addressed in the memorandum titled: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion item # 7 (30-March-2000). Also see comment 8 above.

51. Comment regarding page 33 2nd paragraph: Malaoxon, the active cholinesterase inhibiting metabolite of malathion, was not carcinogenic in male or female rats *in the most recent (1996) study* ***(not owning a positive leukemia finding in males). Recall malaoxon was positive for C-cell tumors in the NCI F344 rat study, both sexes.***

The CARC has determined that MCL was "not treatment related [in the malaoxon rat study] since statistical significance was seen only in males at a dose that was determined to be an excessive dose, there was no dose-response, and the incidences were within the historical control range (15-36%) of the testing laboratory. In addition, the early part of the CARC document stated, "The CPRC agreed with the NTP re-analysis that there was no clear evidence of carcinogenicity due to malathion or malaoxon administration…However, the committee felt that there were many issues regarding the adequacy of each study which needed to be addressed before a firm conclusion regarding the carcinogenic potential of malathion could be made." The new studies do not provide support that the tumors observed in the early studies are treatment related.

52. Comment regarding page 33 last paragraph: It should be noted that the classification of "likely human carcinogen" is based on..... doses not considered excessive. *It is somewhat inconsistent to then say:* it is plausible ..... tumors are induced only at excessive doses, ***without qualifying the reality of the biological significance of tumors in both lower dose groups.***

Based on the outcome of the 12-April-2000 CARC meeting, this entire section will have to be modified.

REFERENCE

Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 9, 2000.**

ATTACHMENT 27

John Carley
Office of the Director
Office of Pesticides Programs

January 27, 2000

As agreed to at the January 13, 2000 meeting, Re your e-mail of January 13, please find below the product of my efforts to collate my comments resident in various memoranda under specific topics of concern. I have attempted to provide a list of "Substance" and a list of "Process". Each topic in the "Substance" list is simply framed, though the background discussion may be lengthy. I am not attempting in this document to add further rationale, points of view, or additional facts to those substantive matters already expressed in the various sources. Rather this document is intended to facilitate the location among the documents, information pertaining to particular subjects. For example, mouse liver tumors have been discussed and written about in many places, so under the topic "Interpretation of mouse liver tumors" various documents are listed wherein this topic in its many and varied aspects have been mentioned. This is essentially a clerical task.

Names (icons) for the various documents appearing on CDs are in bold type, and briefly identified in the attachment.

In the case of "Process", I have first listed certain items relevant to CARC meetings in general, and then provided another list of topics pertaining to malathion which I recommend for external peer review.

I - "Substance"*:*

1) *Interpretation of mouse liver tumors:* **Carc**: pp. 2-3; **Burnam2**: entire memo; **44554901**: p. 5 to end, May 4, 1998 letter to Dr. Jerry Hardisty (attachment 6): entire letter; **Burnam6**: p.3; **Carcrow**: item 8, pp.2-4, item 10, p. 5, item 11, p. 5; **CARC1999**: item viii, p.1; items 2-4, p.2; items 5-8, p.3; etc.; **Rowland**: pp. 3-7, 30.

2) *Interpretation of rat liver tumors:* **CARC1999**: item 9, p.3; **Rowland**: pp.11-12, 30-31.

3) *Interpretation of rat thyroid c-cell tumors:* **C-cell**: entire memo; **Rowland**: pp.2, 15-16.

4) *Interpretation of rat thyroid follicular cell tumors:* **Burnam6**: p.3; **CARC1999**: items 12 and 13, pp.4-5; **Rowland**: p.14.

5) *Interpretation of rat nasal tumors:* **44782301**: pp. 3-4, 11-22; **Bolte**: entire memo; **Burnam10**, entire memo; **Oraltumo**: entire memo; **Burnam5**: entire memo; **CARC1999**: item ix, p.2; **CARC1999**: item 16 and table 16, pp.5-6, item 2-last paragraph, p.6; **Rowland**: pp.x-xi, 12-14, 21, 32.

6) *Interpretation of rat oral tumors:* **Burnamsq**: p.1; **44782301**: pp.3-4, 11-22; **Bolte**: entire memo; **Burnam10**, entire memo; **Oraltumo**: entire memo; **Burnam5**: entire memo; **Rowland**: pp.12-14, 32.

7) *Interpretation of leukemia in the rat:* **Leukemia**: entire memo; **Leukburn**: entire memo; **Rowland**: p.21.

8) *Interpretation of rat interstitial cell testicular tumors:* **Testictu**: entire memo; **Carcrow**:

items 23 and 24, pp. 7-9; **CARC1999**: item 15, p.5; **Rowland**: p.20.

9) *Interpretation of leukemia in the rat (malaoxon):* **Carcrow**: item 25, p.9; **CARC1999**: item ix, p.2; **Rowland**: p.24.

10) *Generic issue - interpretation/use of tumorigenic findings occurring at excessive doses:* **Burnam6**: entire memo; **Leukemia**: pp.2,3; **Leukburn**: pp.2,3; **C-cell**: pp.1,2; **Rowland**: cover memo.

11) *Use of cholinesterase inhibition to conclude dosing excessive:* **Carc**: pp. 1-3; **Carc699**: entire memo; **Carcrow**: item 7, p.1, item 9, p. 4; **CARC1999**: item ix, p.1, item 19, p. 6 last paragraph.

12) *Concerns about nasal tissue pathologic findings in both the combined chronic toxicity/carcinogenicity (neoplastic and non-neoplastic) and inhalation studies (non-neoplastic) where there was no NOEL:* **Carc**: pp.3-5; **Rowland**: pp.21, 28. <u>This is both a CARC and HIARC issue that has not been resolved where the determination of the vulnerability of nasal tissues is concerned.</u>

13) *Absence of NOEL for cholinesterase inhibition in inhalation studies:* **Carc**: p. 5

14) *Collective evidence of tumorigenic findings at low doses:* **Burnamsq**: pp.1-2

<u>II - "Process":</u>

<u>A) Items of General Concern Pertaining to CARC meetings:</u>
1) *Minutes of CARC meetings should be generated for circulation to participants expeditiously, within a specified time frame, while issues are fresh in peoples minds.*
2) *Meeting agenda should be provided to participants within a reasonable time frame prior to meetings. The agenda should indicate the amount of time allocated for discussion of particular topics under review. This agenda should be subject to review and comment by participants, prior to its promulgation.*
3) *The requirement to submit documents to the committee within a specified time frame prior to meetings should not be limited to the presenter. All documents and supporting materials of more than 1-2 pages to come before the committee should be submitted well enough in advance to afford participants the opportunity to become familiar with the subject before listening to the oral discussion at a given meeting. The rendering of full bibliographic citations to reference materials should be considered essential.*
4) *Opportunity should be accorded all participants to pursue follow-up on issues coming before the committee at any given meeting. It should be regarded as self-evident that pursuit of such information may ultimately entail a revisit to a subject already decided, and requests from participants to revisit a subject should be honored.*
5) *Afford full opportunity for participants to express their views without being confronted with claims of constraints of time and the need to move on. The committee as a whole should determine when an individual has exceeded a reasonable opportunity to speak, and encourage written follow-up as the remedy when adequate time to speak is not accorded. Indeed, in the case of the malathion CARC meetings, I recognized early on that faced with the complexity of the subject and inadequate opportunity to speak, it would be necessary that I resort to the written word as a principle mechanism of presenting and preserving information important to the interpretation of the data base.*
6) *Provide in CARC reports the committee's rationale for its decisions, e.g. in the case of*

malathion, why statistically significant evidence of thyroid c-cell carcinogenicity was discounted, and how CARC's decision differs from the alternative interpretation, and not simply recording the vote.

B) The following issues concerning malathion are recommended for submission to SAP, or other external peer review, to address the expressed differences in reasoning between myself and CARC.

1) *Mouse liver tumor response.*
2) *Thyroid c-cell tumorigenic response in the rat.*
3) *Thyroid follicular cell tumorigenic response in the rat.*
4) *Interpretation of evidence of leukemia in the rat under OSTP (1985)'s definition of carcinogen.*
5) *Interstitial cell testicular tumor response in the rat.*
6) *Interpretation of rat nasal tissue histopathology and tumorigenic response in the rat.*
7) *Adequacy of oral cavity assessment for tumorigenic response, and CARC's conclusion regarding squamous cell tumorigenic response.*
8) *Concerted evidence of tumorigenicity (several endpoints) in low dose groups. For example, are the low dose hepatocellular tumorigenic responses in the mouse and rat mutually supportive?*
9) *Decisions to discount dose levels as excessive for carcinogenicity assessment based on cholinesterase inhibition. Inherent in such review would be the precedent for the decision, existence of guidelines, which forms of the enzyme must be inhibited and by how much, and so on.*
10) *Acceptability of OSTP's (1985) definition of carcinogen, and if considered acceptable, the rigor of its application in CARC's interpretation of the malathion studies.*
11) *Interpretations of tumorigenic findings or the absence (or reduced incidences) of the same, at doses considered excessive.*
12) *Application of general principles of competing toxicity and increased mortality in mitigating expression at excessive doses of a tumorigenic dose-response occurring at acceptable lower doses.*
13) *Acceptability of the combined chronic toxicity/carcinogenicity study to evaluate carcinogenic potential in male rats.*
14) *Adequacy of Q* method to address risks posed by low dose tumor findings, e.g., liver tumors in the female rat at 100/50 ppm, in the absence of a NOEL.*

Reference:
Office of Science and Technology Policy (OSTP)(1985): Chemical Carcinogens: a Review of the Science and Its Associated Principles, February 1985. *Fed. Register*, 50 (No. 50), 10772-10442.

Brian Bementi, Ph.D., D.A.B.T.
Senior Toxicologist
Toxicology Branch/HED

Attachment (1)

Attachment

Identification of Names (CD icons) cited:

44782301: May 27, 1999 review of rat nasal tissue histopathology re-assessment
44554901: December 1, 1998 review of Pathology Working Group (PWG) on mouse liver tumors
Bolte: November 18, 1999 review of correspondance with Dr. Henry Bolte, under cover memo of December 7, 1999 memo to William Burnam
Burnam2: May 29, 1998 memo to William Burnam
Burnam5: July 22, 1999 memo to William Burnam
Burnam6: November 12, 1999 memo to William Burnam
Burnam10: January 12, 2000 memo to William Burnam
Burnamsq: September 21,1999 memo to William Burnam
Carc: November 26, 1997 memo to William Burnam
CARC699: June 21, 1999 memo to William Burnam
CARC1999: October 6, 1999 memo to Jess Rowland
C-cell: May 18, 1999 memo to William Burnam
Carcrow: April 1, 1999 memo to Jess Rowland
Leukburn: April 27, 1999 memo to William Burnam
Leukemia: February 24, 1999 memo to CARC
Oraltumo: July 13, 1999 memo to William Burnam
Rowland: October 28, 1999 memo to Jess Rowland, accompanied by October 28 draft CARC report, with my comments in margins
Testictu: June 7, 1999 memo to William Burnam

ATTACHMENT 28

From: Brian Dementi 02/03/2000 2:37 PM
To: John Carley
cc: Dwight Welch, John Hirzy
Subject: Summary of malathion CARC issues

On January 27, I submitted to you a memorandum on the indicated subject. This required all of my time since we agreed to perform the task by January 28. I confirm this to be a complicated task. To prepare the January 27 letter it was necessary that I read all of my memoranda and CARC draft reports in order to identify issues and then collate the same in the form of the letter provided. Once engaged in this task, and then realizing it was too ambitious, I discussed the matter with Dwight Welch and Bill Hirzy suggesting we give you a call to say if pursued the project may compromise that which I have already accomplished.

We agreed to strive to fulfill the commitment, to state the issues and identify the various documents wherein the topics were discussed. It is my opinion that the differences of opinion you would have me identify (i.e. "I think x"; "the committee thinks y") are to be found within the documents as cited for each topic. However, toward assisting you further, I have added language to items rendered in II B of my January 27 letter that will set forth my fundamental differences of opinion with respect to those of the committee on those issues. In seeking to thus help HED, please understand my uneasiness in attempting to embrace or craft into very short sentences that which is developed in much background information. Persons interested in these subjects must examine the cited documents for factual information and the rationale.

1) Mouse liver tumors:

a) positive liver tumorigenic response across all doses, i.e. no NOEL for males, and positive at the top two doses in females. The finding extending to the lowest dose in males, not unlike the liver tumorigenic response in the female rat in this respect, should be regarded as of particular concern;

b) CARC should not leave unexplained the more remarkable liver tumorigenic responses, particularly in females, in the more recent study, versus findings in the earlier NCI study which the new study was designed to replicate at the top two doses.

2) Thyroid c-cell tumorigenic response in the rat: the finding is positive among male rats across the 0-500 ppm dose range, and cannot be discounted as CARC has done by findings at higher "excessive" doses, lest the study be considered unacceptable for evaluation of this tumorigenic response. Findings at low doses should be of particular concern and discounted only by the most persuasive forms of evidence.

3) Thyroid follicular cell tumors: competing toxicity and increased mortality among male rats at 6000 ppm and 12000 ppm (dose levels considered as excessive by CARC) may have dampened or compromised full expression of a tumorigenic response at these

higher doses already evident in the existing data set, i.e. a positive dose trend (p = 0.035) and a nearly positive (p = 0.077) pairwise comparison for the 6000 ppm dose group. I challenge, therefore, CARC's conclusion that the study can be accepted as a negative study for this tumorigenic response. In my view (not stated as such previously, though evident in the reasoning) this tumorigenic response should be viewed as suggestive evidence of carcinogenicity that cannot be discounted because of the unacceptability of the study in male rats at the high dose levels, which CARC itself has called excessive. This is a difficult interpretation which I feel merits an external review.

4) Interpretation of evidence of leukemia in the rat under OSTP (1985)'s definition of carcinogen: evidence of a dose related increased incidence of mortality attributed to leukemia among male rats diagnosed with leukemia constitues positive evidence of carcinogenicity under the second aspect of OSTP's definition of carcinogen, namely, ".....or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen." (pp. 10410-10415). I contend the dose-related increased mortality (where mortality itself indicates a more advanced stage) is evidence of a dose-related increased rate of development of leukemia. It could be argued that rats harboring leukemia are simply more susceptible to early death due to the increasing secondary toxicologic burden of the test material, but to confirm that possibility and to discount the possibility of a direct compound effect in development of the response, the mechanism would need to be established. I am not aware CARC has provided a rational response to this issue.

5) Interstitial cell testicular tumor response in the rat: Statistical treatment of this tumorigenic response was positive across all four doses as presented in the study report, and was positive across the top three doses as analyzed by the Peto test within HED. I accept these assessments as showing a dosing related higher incidence than expected of this tumorigenic response, and hence, as a positive carcinogenic effect by recognized definitions of a carcinogen. In my view the Peto test, as required by the CARC, was conducted in the prescribed manner by HED's statistician, and was positive. I am not satisfied with CARC's rationale (absent mechanistic data) for discounting this response, and would desire another expert opinion.

6) Interpretation of rat nasal tissue histopathology and tumorigenic response in the rat: I accept as evidence of carcinogenicity all four extremely rare nasal tumors, two in males and two in females, at the top two dose levels. CARC discounts the findings in males, as I understand, because dosing was excessive, but again, as with certain other tumor types, to the extent tumorigenic findings are discounted in high dose groups, the study in my view is unacceptable in males. The issue is complicated by evidence of nasal histopathology in the long term combined chronic toxicity/carcinogenicity studies in the F344 rat for both malathion and malaoxon, and in the dose range-finding and subchronic inhalation studies of malathion in the rat. While a new inhalation study is being required, I am not satisfied that CARC has an adequate interim handle on risks posed with respect

to the nasal mucosa, particularly by the inhalational route of exposure. Nasal tissue vulnerability is an important and unresolved issue at this time.

7) Adequacy of oral cavity assessment for tumorigenic response, and CARC's conclusion regarding squamous cell tumorigenic response: I contend the four extremely rare squamous cell tumors (three in females, one in males) appearing in oral mucosal tissues in the malathion combined chronic toxicity/carcinogenicity study in the rat cannot be discounted as evidence of carcinogenicity. Furthermore, as these tumors were identified in but a partial and inadequate assessment of oral cavity histopathology, there is a greater encumbancy to accept these as real until an adequate histopathology assessment of the entire oral cavity tissues has been performed. I have suggested this need for additional histopathology to CARC, and am concerned the registrant did not of his own volition follow-up with a complete oral cavity histopathology assessment once these tumors were found. CARC discounted the oral tumors at one meeting on the grounds these tumors are not as rare as the nasal tumors. However, subsequent follow-up information, in my opinion, demonstrates the squamous cell tumors to be essentially as rare as the nasal tissues in various data bases. Am not aware CARC has responded to the more recent information. I have not been availed of the latest, or final, CARC report.

8) Concerted evidence of tumorigenicity (several end points) in low dose groups. For example, are the low dose hepatocellular tumorigenic response in the mouse and rat mutually supportive? I have expressed concern over certain tumorigenic responses that appear to extend into the low dose range, incorporating in certain cases even the lowest dose, absent a NOEL (e.g. male mouse liver tumors, female rat liver tumors, leukemia in male rats as defined above, extremely rare oral squamous cell tumors, possibly testicular tumors). My concern is whether collectively these speak more strongly of a low dose biological effect, than any standing alone, and whether CARC has adequately addressed this possibility in its assessment.

9) Decisions to discount dose levels as excessive for carcinogenicity assessment based on cholinesterase inhibition. Inherent in such review would be the precedent for the decision, existence of guidelines, which forms of the enzyme must be inhibited and by how much, and so on: I do not accept the view that cholinesterase inhibition (absent any guidelines or rationale) alone, absent cholinergic clinical signs, can be cited as adequate rationale to discount a dose level in question as excessive, and in so doing discount remarkable tumorigenic findings observed at that dose level. In my view, inadequate rationale has been provided by CARC to justify dismissal of dose levels as excessive, and in so doing precluding testing at high doses (MTD) called for in cancer bioassays.

10) Acceptability of OSTP's (1985) definition of carcinogen, and if considered acceptable, the rigor of its application in CARC's interpretation of the malathion studies: The OSTP (White House Office of Science and Technology Policy) definition reads as follows: "A chemical carcinogen may be a substance which either significantly increases the incidence

of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen." (pp. 10414-10415) I have sought from CARC its views as to the veracity of this definition of a carcinogen, but my question has not been acknowledged or addressed. I posed the question because it seemed to me that on certain of the tumorigenic end points, the committee appeared too focused on the first element of the definition (strict statistical treatment of tumor incidence) to the neglect of second element (rate of tumor development). Evidence of enhanced tumor development, including such findings as greater proportions of malignant versus benign tumors, tumor multiplicity, tumor size, decreased tumor latency, etc, may not yield statistical evidence of carcinogenicity, but yet constitute positive evidence of carcinogenicity according to the OSTP definition. If CARC owns this definition, then it should provide more evidence of its utilization in the interpretation of the end points at hand.

11) Incorporation of tumorigenic findings or the absence (or reduced incidences) of the same, at doses considered excessive: Questioned here is the use of tumorigenic findings, or the absence of the same, in a dose group considered excessive by CARC. A prime example is CARC's use of the top two dose groups (6000 ppm and 12000 ppm), considered excessive doses by the committee, for assessing tumorigenicity among male rats, in the combined chronic toxicity/carcinogenicity study in the rat. I contend as improper the discounting of tumorigenic findings of one type at a dose level considered excessive, while utilizing decreased tumorigenic findings of another type in these excessive dose groups to discount positive findings at lower doses considered by the committee to be acceptable. By contrast, I contend that accepting tumorigenic findings at excessive doses is more defensible than accepting as negative a study without findings at excessive doses.

12) Application of general principles of competing toxicity and increased mortality in mitigating expression at excessive doses of a tumorigenic dose-response occurring at acceptable lower doses: In my opinion, having cited authoritative sources, competing toxicity and increased mortality at excessive doses may diminish or even preclude tumorigenic responses identified at lower doses. Furthermore, in consideration of the potential for such compromises of tumor expression to occur at excessive doses, negative or diminished findings at such doses cannot be accepted as negative evidence of carcinogenicity. It is more acceptable, as I understand, to accept positive findings at excessive doses unless (according to EPA's draft Cancer Guidelines) it can be shown such tumorigenic responses resulted from toxicity as opposed to tumorigenicity of the test material. I am not satisfied CARC has made proper use of these concepts, specifically, in discounting certain tumorgenic findings at dose levels considered excessive without demonstrating these arose secondary to toxicity, while on the other hand accepting diminished tumorigenic responses at excessive doses as negative evidence. There have been no statements from CARC clarifying its philosophy.

I must add that in the case of liver tumorigenic responses in female rats in the combined chronic toxicity/carcinogenicity study with malathion, I concur with CARC's interpretation at all doses, including that for the highest dose group, as being consistent with my understand of the principles at issue here, i. e., there is no evidence the tumorigenic response observed at the highest dose, the only dose level considered excessive in females, was due to anything other than the tumorigenicity of the test material.

13) Acceptability of the combined chronic toxicity/carcinogenicity study to evaluate carcinogenic potential in male rats: I have difficulty accepting CARC's decision concerning acceptability of the study as essentially a negative study in male rats, specifically the male F344 rat. Discounting the top two doses as excessive, and accepting the lower dose levels, in my opinion precludes testing at adequately high dose levels. The findings suggest the need for another dose group somewhere between the 500 and 6000 ppm dose groups. It may be the F344 rat is a poor model due to competing toxicity. On the other hand, if CARC accepted the study as demonstrating tumorigenic findings in males at the lower doses, perhaps that would be the end of it. Given the male rat assessment is thus confounded, greater reliance must be placed on findings in females, i.e., as carrying more weight than a single gender finding.

14) Adequacy of Q* method to address risks posed by low dose tumorigenic findings, e.g., liver tumors in the female rat at 100/50 ppm, in the absence of a NOEL: This is a philosophical question raised by me that has not been discussed at any of the CARC meetings as I recall. It is my concern that to the extent low dose tumorigenic findings occur at more elevated incidences than expected based on those incidences at much higher doses (e.g. the female rat liver tumor response), for whatever reason, such as a change of mechanism across the dose range, can the Q* calculation, employing all doses, be expected to address risks posed at the low dose level. I am concerned low dose findings in this assessment, close to those that minimally inhibit cholinesterase are of peculiar concern to the public health, and petition for additional expert comment on the utility of the Q* method to deal with this. This is more a gut feeling than one borne of any particular expertise or evidence I bring to the table. The Q* method has been used by CARC to address public health risks based on the female liver tumorigenic response.

I trust these comments taken in concert with those in the January 27 letter come closer to that which you were seeking. Please be advised I have not seen CARC's final report. I should offer my availability to discuss these topics with any interested individual or group within HED who may be reviewing the subject.

ATTACHMENT 29

Mr William Burnam April 27, 2000
Chairman
Cancer Assessment Review Committee
HED/OPP

As you know, on March 20, 2000 Cheminova A/S submitted (through Jellinek, Schwartz & Connolly, Inc.), a company initiated Pathology Working Group (PWG) Report (MRID 45069401) on the female rat liver tumor response for the 1996 Combined Chronic Toxicity/Carcinogenicity study with malathion in the F344 rat (MRID 43942901). This submission was reviewed March 28 by HED's consulting pathologist, Dr. John Pletcher, and received a preliminary review by Drs. Marion Copley and Brian Dementi on March 31. The PWG report and the two reviews were taken under advisement by the Cancer Assessment Review Committee (CARC) at its meeting on April 12.

Inasmuch as time did not permit a complete and more thorough review by Dr. Copley and myself before the April 12 CARC meeting, the following comments constitute an expression of my assessment, now having had more opportunity to adequately examined the PWG submission and related information. These comments derive from an evaluation of the following sources of information:

a) the PWG submission;
b) a follow-up set of questions faxed on April 7 to Dr. Jerry Hardisty, PWG Chairman and his response, faxed April 10;
c) my personal notes/recollections of a meeting with Dr. Hardisty and others held April 11 in HED, at Dr. Hardisty's request;
d) reviews of relevant background materials, including:
i) a publication by Goodman et al (1994) submitted appended to the PWG report as supporting documentation setting forth criteria which served as the bases for the PWG's histopathology diagnosis,
ii) a publication by Eustis et al.(1990), pertaining to the interpretation of liver tumorigenic response in the Fischer rat, and
iii) EPA's draft Cancer Risk Assessment Guidelines (July, 1999).

In the March 31 preliminary review by Drs. Copley and Dementi, certain issues were addressed, summarized as follows:

1) Discrepancies noted between assertions in the Registrant's cover letter and the PWG report were noted;

2) The purpose of the PWG under PR Notice 94-5 is to diagnose (re-diagnose) pathology slides. The PR Notice does not require or call for any discussion of the relevance, for purposes of risk assessment, of the hepatic neoplasms which occurred in the study, as indicated in the PWG report (p. 10);

3) The CARC will independently determine what tumor and non-neoplastic values are most appropriate for assessment of liver tumorigenicity, and the regulatory implications of the PWG re-reads;

4) The results of the study pathologist and peer reviewing pathologist were employed by the PWG Chairman in selecting slides to be submitted on March 15 to the full PWG, namely, findings of carcinoma, adenoma, hepatocellular alteration of at least moderate degrees of severity and hypertrophy/hyperplasia.

Questions posed in the preliminary review included:

1) Should the signatures of the five PWG pathologists on page 5 be interpreted as their approval for the entire report?

2) Was Dr. Henry Bolte present, and if so, what was his role in the pathology peer review performed March 14 by Dr. William Busey?

3) Did the peer review pathologist (Dr. Busey) have access to the doses and the diagnoses of the study pathologist?

4) Can a reviewing pathologist reliably evaluate as many as an estimated 625 sections in one day?

5) What was the level of review performed by the peer reviewing pathologist?

6) Why were certain adenomas identified by both the study pathologist and the reviewing pathologist, rendered as “no corresponding diagnosis” by the PWG?

7) Foci of cellular alteration are often considered pre-neoplastic and part of the neoplastic continuum. How do these lesions, referred to as hepatocellular alterations in this study, figure into PWG’s diagnoses of hepatocellular pathology?

8) Given the remarkable concordance between the initial diagnoses of the study and reviewing pathologists, including agreement on four of the five carcinoma diagnoses (where the reviewing pathologist called the fifth an adenoma), what was seen microscopically at the PWG that by contrast resulted in the downgrading of all carcinomas, i.e. what are the contrasting pathologic or histologic descriptors that justify the revised diagnoses? We accept that Dr. Busey was employing in his peer review on March 14, the same criteria employed by the PWG on March 15. Such explanation should have been recorded in a “comments” column in the PWG report.

9) If the first two pathologists agreed on a tumor diagnosis, which was subsequently changed by the PWG, does this suggest a fine line distinction?

10) If new criteria of classification are being applied in this study of malathion, what does the panel say regarding relevance of the historical control data base, from both NTP and the testing facility?

There are yet other questions time does not permit addressing. It should be apparent that in the face of so many questions, a completed review of the PWG report to replace the March 31 preliminary review was not possible before the April 12 CARC meeting, given that efforts to resolve the questions were proceeding as late as April 11 in our interview with Dr. Hardisty, in addition to the weight of other matters.

In the following passages I will attempt to present answers to the above and certain other questions HED subsequently posed, as received from Dr. Hardisty in both his written and oral communications, and to present an independent expression of the concerns I have regarding the performance of this PWG and interpretation of the findings.

Responses to questions

1) Should the signatures of the five PWG pathologists on page 5 be interpreted as their approval of the entire report? Dr. Hardisty has responded in the affirmative.

2) Was Dr. Henry Bolte present, and if so, what was his role in the pathology peer review performed March 14 by Dr. William Busey? Dr. Hardisty has affirmed Dr. Bolte's presence, as the performing laboratory's host, but that he had no role in the PWG.

3) Did the peer review pathologist (Dr. Busey) have access to the doses and the diagnoses of the study pathologist? The answer appears to be yes, that Dr. Busey examined all slides to identify any additional lesions, but also his objective was to review initial diagnoses by the study pathologist.

In my view, an ideal peer review would involve complete diagnoses of all slides, not knowing the study pathologist's diagnoses, such that two independent sets of diagnoses of all slides would be available for resolution by the PWG. It is somewhat surprising that on p. 5 of Dr. Hardisty's April 10 fax he says: "Differences of opinion in diagnosis between the study pathologist's initial diagnoses and the PWG consensus diagnoses are included in the tabulation in Appendix A. These differences resulted from the reexamination of the slides using the criteria included on pages 14 and 15 of the PWG report.", while glossing over Dr. Busey's peer review diagnoses, performed by the same criteria, and yet in remarkable concurrence with Dr. Bolte's diagnoses.

4) Can a reviewing pathologist reliable examine so many slides in one day? Dr. Hardisty responds clearly in the affirmative in his faxed response (pp. 3-4), and he explained the utility of EPL's automated process more fully on April 11.

5) What was the level of review performed by the peer review pathologist? He examined all slides, and according to the text of the PWG report, employed the same criteria (Goodman et al 1994) (p. 5 of the PWG report) as employed by the PWG.

6) Why were certain adenomas identified by both the study pathologist and the reviewing pathologist rendered as "no corresponding diagnosis" by the PWG? There were three such instances, namely, rat #s 2554, 4531 and 5528. In his April 10 fax, Dr. Hardisty says the diagnoses (now changed to hepatocellular alteration) were recorded in the table across from this same finding rather than across from the original adenoma call. However, on April 11, he explained "hepatocellular alteration" should have been entered rather than "no corresponding diagnosis" across from the original adenoma diagnosis, since hepatocellular alteration was also diagnosed by the first two pathologists (and confirmed by the PWG) as a secondary lesion. In other words, in downgrading the adenoma, there then existed two hepatocellular alteration diagnoses (i.e. multiplicity for this lesion) for the three rats in question, which should have been reflected in the table. I should note that multiplicity of preneoplastic lesions, such as hepatocellular alteration, should be tabulated for this study.

7) How do hepatocellular alterations figure into PWG's diagnoses of hepatocellular pathology? I believe it is fair to say that both Dr. Copley and I feel that to the extent the PWG elected to discuss carcinogenicity, which is not an element required under PR Notice 94-5, that it would have been informative for the PWG pathologists to comment on the role of hepatocellular alterations in the neoplastic process, and to have included final incidences of hepatocellular alterations (of at least moderate degree of severity) along with adenomas and carcinomas in tables 2 and 3 of the PWG report.

In his faxed response (p. 5), Dr. Hardisty in essence says the intent of the PWG review was to identify neoplasms. Non-neoplastic lesions were examined only insofar as these might be reclassified as neoplasms by the PWG. In our discussion with Dr. Hardisty on April 11, he explained he forwarded to the PWG for review on March 15, only those hepatocellular alterations diagnosed by the study pathologist or peer review pathologist as of at least moderate degree of severity. Of course, neoplasms previously diagnosed were similarly forwarded to the PWG. Dr. Hardisty explained that he selected for PWG examination only hepatocellular alterations of <u>moderate degree of severity, because these were the most likely to be reclassified as adenomas</u> by the PWG.

I do not know what precedent there is for excluding from PWG, incidences of hepatocellular alteration of severity rated less than moderate. I should note at this point that, in my view, inherent in this very observation is affirmation of the possibility of a continuum between hepatocellular alteration and adenoma, and that at least in the opinion of the Chairman, others might consider these to be adenomas. Also, in his faxed response, Dr. Hardisty goes on to say: "The PWG was not asked to address the significance, if any, of the nonneoplastic findings in the liver." I would introduce my observation at this point that to the extent the

PWG entered into a discussion of carcinogenicity (not called for in the PR Notice) which included evaluations of such data as mortality, body weight, organ weights, etc, the assessment should have included comment on incidences of hepatocellular alteration, as this lesion is considered by many experts to be a preneoplastic lesion, and is probably the reason the PWG examined those of at least moderate degree of severity.

8) What was seen microscopically at the PWG that resulted in the downgrading of carcinomas to adenomas, or adenomas to hepatocellular alteration?

I feel this question is justified for the benefit of non-pathologists, such as myself, who participate in the review. This is particularly true because, for example, where carcinoma diagnosis is concerned, HED's consulting pathologist, Dr. Pletcher, says that the well defined criteria under the STP Guidelines, make the differentiation of carcinomas from adenomas relatively easy for a pathologist with rodent experience. Furthermore, in support of this, Goodman et al (1994) claim that in terms of microscopic appearance: "Hepatocellular carcinomas generally have characteristic histologic features readily (emphasis added) distinguishing them from other primary and secondary liver tumors." [p. 4 (110)] Given this apparently well recognized ease of distinguishing carcinomas, again what explains the incorrect diagnoses of both Drs. Bolte and Busey, prior to the PWG?

The PWG sets forth on pages 14-15 the diagnostic criteria (based upon Goodman et al 1994). Three criteria are provided for carcinoma. If Drs. Bolte and Busey concurred that a certain lesion was carcinoma, while the PWG changed the diagnosis to adenoma, it would be helpful for the PWG report to say something to the effect in terms of the stated criteria, for example, that while Drs. Bolte and Busey made their diagnoses based upon trabecular formations, the PWG by contrast concluded these were not sufficiently well developed to designate carcinoma. However, no such answer was provided by Dr. Hardisty in his faxed response to question 3) d) (p. 6). On April 11, Dr. Hardisty had to leave before I could pursue this question further. What is the benefit, one might ask, in presenting the diagnostic criteria to be followed in this PWG, if those outside the PWG are provided no examples of how the criteria were employed, or could be used, to explain diagnoses that were revised?

9) Was there a fine-line distinction between the initial diagnoses and the PWG diagnoses? This question is really an extension of question 8, i.e. in a graded response or continuum of tumor development, where is the line drawn. I would be pleased to know, for example, in those cases wherein adenoma was downgraded to hepatocellular alteration, how close was the determination in terms of the stated criteria. In his response to question 3) e), Dr. Hardisty speaks in the generic sense of the great value of the PWG in providing a forum in which to resolve diagnoses for the lesions that are borderline in nature. I am sure we would all agree with this philosophy, and appreciate the very great merit for this reason in performing a PWG. Yet, he did not address the question of whether the very lesions revised in the current PWG were borderline. I would be curious to know, for example, whether perhaps one peculiarity of the original carcinoma

diagnoses resulted in all four being downgraded, and likewise for the downgrading of adenomas. We feel as though we have proffered a reasonable question, but have not been graced with a response.

10) If new diagnostic criteria were employed, what does this say as to the relevance of the historical controls? Dr. Hardisty responds that it has no effect on relevance of NTP's data base because it has been peer reviewed. He is not certain about the testing laboratory. He says that: "It is unlikely that significant changes would be made to the testing facilities historical control data even if reviewed using the criteria published by Goodman et al. I would have to express some personal concern with this view, given the substantial changes of diagnoses that result from the current PWG. Also, I am not certain that NTP's data base has been fully contemporized under Goodman et al, i.e. all studies. On April 11, Dr. Hardisty indicated the criteria have been the same since 1984, as I understood him to say. If that is true, what is so particularly meaningful in citing Goodman et al?

Summary of Concerns Regarding the Pathology Working Group (PWG) for Malathion

1) While observers representing the registrant were present at this March 15, 2000 PWG meeting, there were none present from the Agency. I should note that when CARC was requiring a PWG for the mouse study, I sought advice from the National Toxicology Program's Dr. Robert Maronpot on the conduct of PWGs. He responded in a letter dated July 24, 1997, copy appended. You will note in his letter the various suggestions he offered. I should note in particular that he said: "It would also be beneficial to have yourself or another EPA toxicologist participate in the peer review process as an observer to insure that all important questions you might have are resolved. Again, I stress the importance of reviewing all liver tissues, even from animals without diagnosed liver tumors, since additional neoplasms may be found and preneoplastic lesions (emphasis added) of the liver may be documented in some mice without overt liver tumors."

I should note that examples of the types of questions one might pose include those such as: In terms of diagnostic criteria, where does the PWG draw the line between hepatocellular alteration/adenoma and adenoma/carcinoma? In pathologic terms, what explains on individual slides from the malathion study changes of diagnosis by PWG, versus earlier diagnoses by the study and reviewing pathologists? How would the PWG characterize the severity of hepatocellular alteration in the three cases downgraded from adenoma in the penultimate dose group? Should there be qualifying remarks explaining changes of diagnoses? and so on.

Also, one might have suggested the PWG tabulate along with adenomas and carcinomas, incidences of hepatocellular alterations of at least moderate degree of severity, as these were forwarded to the PWG by the Chairman. This latter suggestion is important in light of the

emphasis placed on preneoplastic lesions in Dr. Maronpot's letter, and would be important information to consider as "key events" in the analysis of the overall tumorigenic response under EPA's draft Cancer Risk Assessment Guidelines. However, this opportunity to have an EPA observer present was missed, in that I was not even informed of the conduct of the present PWG on the rat study until the day it was being performed, even though it was known beforehand to OPP, as I understand.

2) I would desire more information from experts in the field as to how much time would be required for an expert pathologist to perform a thorough and reliable evaluation of as many as perhaps 620 liver histopathology sections from a chronic bioassay.

3) The lack of ranking as to severity of "hepatocellular alteration" diagnoses by PWG, though perhaps not a requirement under the PR Notice, makes it difficult for EPA to distinguish and estimate the final incidences of those which might be of greater concern, i.e. those of the variety of at least moderate degree of severity that were selected for referral to the PWG. This is important because according to EPA's Cancer Guidelines, incidences of "key events" in the neoplastic process should be evaluated along with tumor incidences in evaluating the carcinogenic response. (See pp. 3-1 to 3-2, 3-5 to 3-7, 3-11 to 3-12, 3-15 to 3-16 of the Guidelines). It may well be that moderate to large hepatocellular alterations would play a significant role in the interpretation, particularly if these are qualitatively the same as adenomas (as information in Goodman et al 1994 indicates), but not quite robust enough in the minds of the PWG pathologists to be designated as neoplasms.

4) The PWG report says: "The purpose of the PWG review was to determine the incidence of hepatocellular neoplasms in female rats following currently accepted nomenclature and diagnostic criteria (Goodman DG, et al., 1994)." (p. 8). Actually, the PWG is not intended to be narrowly focused on incidences of neoplasms. I think this is supported by Dr. Maronpot's letter where he places importance upon identification of preneoplastic lesions in animals without overt liver tumors. Furthermore, the PR Notice 94-5 itself addresses this as follows: "For any target tissue which is being re-evaluated, all slides containing that tissue in all dose groups, as well as the controls, must be re-read by the peer review pathologist. This is to include slides previously classified by the study pathologist as within normal limits, in addition to those having tumors, hyperplasia, hypertrophy, foci of cellular alteration or other non-neoplastic lesions." Where hepatocellular alterations are concerned, the PWG report indicates that only those of "moderate degrees of severity" were referred to the PWG pathologists. (p. 10).

I should note that the March 20 covering letter of Ms Diane Allemang for the submission of the PWG report, is incorrect in saying: "The purpose of the PWG was to validate the accuracy and consistency of the initial histopathology examination for the diagnosis of proliferative lesions in the liver of female rats. The reviewing pathologist (Dr. Bill Busey) reexamined all sections of the liver from all animals in all control and treated groups for

female rats. Following Dr. Busey's review, the PWG (a panel of expert pathologists) was convened to examine tissue sections (including all (emphasis added) foci of cellular alteration, all hypertrophic/hyperplastic lesions, and hepatocellular neoplasms) from the liver from female rats diagnosed in the final study report and/or by the reviewing pathologist."

To the extent that all hepatocellular alterations were not examined by the PWG, the Agency does not have an assessment of PWG confirmed incidences of this lesion to be used as "key events" along with neoplasms in evaluating the neoplastic response under EPA's Cancer Guidelines. Lacking such data on all hepatocellular alterations, there would be incumbency to include in the analysis as "key events", incidences of those lesions considered severe enough to be confirmed by the PWG, which would include those resulting from the PWG's downgrading of adenomas.

5) The PWG Report indicates that "The lowest-effect level for hepatocellular neoplasms (emphasis added) in female rats in this study was considered to be 12,000 ppm." (p. 9) Unfortunately, presentation of the revised diagnoses in the PWG report does not permit determination of the lowest-effect level for the incidence of hepatocellular alteration of "moderate degree of severity", nor as indicated previously is incidence data for the latter lesion available in the PWG report for use in the interpretation of the overall neoplastic response under EPA's Cancer Guidelines.

The Guidelines indicate that: "Identification of a key event does not imply that it is adverse in itself, only that it is an observable step preceding tumor development." (p. 3-12) There is no evidence in the Guidelines to suggest that in order to identify a particular event as a key event, all expressions of such events are on an obligate course to neoplasia; some or even most may reverse. As mentioned in the Guidelines, hyperplasia may be viewed as a key event (p. 3-7), yet, all hyperplasias do not necessarily become neoplasms. I prefer to think of such preneoplastic lesions as constituting a population of events among which there is enhanced probability of neoplasms developing, versus that of normal liver.

The Guidelines also say: "If confidence is high in the linkage of a precursor effect and a tumor effect, the assessment of tumor incidence may be extended to lower dose levels by linking it to the assessment of the precursor effect (Swenberg et al. 1987)." (p. 3-17) I should also cite in support of the view that foci of hepatocellular alterations are important in this context, the work of Eustis et al (1990), in which the "natural history of neoplasia" for Fischer rat liver tumorigenic response is considered to consist of the progression: foci of cellular alteration > adenoma > carcinoma. I believe this concept finds support both in Goodman et al (1994) and in EPA's Guidelines.

In this particular case concerning hepatocellular tumorigenic response in female rats following malathion treatment, this reviewer, for the reasons indicated, concludes confidence is high in the linkage of hepatocellular alteration of moderate degree of severity as a precursor effect with liver adenomas. This reviewer also concludes that the downgrading of

carcinomas to adenomas (and not another lesion), and adenomas to hepatocellular alteration (and not another lesion) is supportive of, and consistent with, published works that note in the case of hepatocellular tumorigenic response, a "natural history of neoplasia" which is characterized by foci of alteration > adenoma > carcinoma, and that the downgrading in this study of carcinoma > adenoma and adenoma > hepatocellular alteration, may constitute little more than a frame shift toward a less severe response, borne of application of more strict criteria imposed by this PWG, and should not be interpreted as some sort of affirmation that Drs. Bolte and Busey were incorrect in their initial assessments. It may simply be a matter of degree.

In support of the concept of a natural history of neoplasia for hepatocellular lesions of liver epithelium, while not referring to the "natural history of neoplasia" as such, Goodman et al (1994) describe the characteristics of foci of cellular alteration, adenoma and carcinoma, as if these represent a continuum. Differences between foci of cellular alteration and adenoma appear to reside primary with lesion size and degree of compression. In fact Goodman et al (1994) say: "Such compression is not a prominent feature as it usually is with hepatocellular neoplasms, and this, as well as the preservation of hepatic lobular architecture are the main morphologic differences between foci and hepatocellular adenomas." (p. 2) These authors also say: "Except for occasional cellular atypia, the cells in adenomas are comparable morphologically to those described for foci of cellular alteration." (p. 3) According to these authors, hepatocellular carcinomas, ranging in diameter 1 cm to over 10 cm, tend to be larger than adenomas (few mm to several cm).

Goodman et al also say: "Hepatocellular carcinomas generally have characteristic histologic features readily (emphasis added) distinguishing them from other primary and secondary liver tumors. Except in anaplastic tumors, the individual cells generally approximate the appearance of normal hepatocytes. They are typically cuboidal with abundant cytoplasm and contain a centrally placed nucleus. The tinctorial appearance of the cytoplams may be eosinophilic, basophilic, clear, or a mixture of types. Cells often resemble cells found in foci of cellular alteration or adenomas (emphasis added)." (p. 4) The text goes on to describe certain patterns neoplastic hepatocytes may form within the neoplasm, e.g. "trabeculae", "acinar or glandular form" or a "solid pattern", each of which has its descriptors. The point is that these three types of lesions, foci of hepatocellular alteration, hepatocellular adenomas and hepatocellular carcinomas, appear to be recognized by Goodman et al as a continuum in hepatocellular epithelial tumorigenic response. Thus all three findings properly should be considered in assessing hepatocellular tumorigenic response to a test material.

In the data set coming out of the PWG, adenoma incidences are: 0, 1, 1, 0 and 5 for dose groups I through V, respectively. Incidences of hepatocellular alteration of moderate degree of severity, as best estimated from data in the PWG report are: 0, 4, 3, 6 and 5, in the same respective order. It should be noted that the incidences for both of these lesions in the control group is zero.

Now, if one takes an approach advocated in EPA's guidelines, and links these data, combined incidences would be: 0/55 (0%), 5/55 (9.1%), 4/55(7.3%), 6/55(10.9%) and 10/55(18.2%) for groups I through V, respectively. This data should stand as evidence of a positive tumorigenic response across all doses, until established by more definitive data; as revealing a positive dose-response relationship for carcinogenicity; as positive at all doses; and as absent a NOAEL.

As stated earlier, since only hepatocellular alterations of moderate degree of severity were forwarded to the panel of pathologists, this PWG may be deficient in reference to the PR Notice, and inadequate to provide any support for alternative conclusions. A comment is indicated here. The hepatocellular alterations and tumors making up this composite represent the more outstanding findings among the lesions observed. Insofar as the hepatocellular alterations that were referred to the PWG were among the more robust forms, these may indeed be examples from among all hepatocellular alterations that are emerging, and should more logically be combined with those having already reached the adenoma stage. Indeed, in our conversation with Dr. Hardisty on April 11, he indicated his reason for selecting out the hepatocellular alterations in the "moderate stage of severity", was borne of his concern the PWG might classify these as adenomas. Thus, there may be evidence here of a break between hepatocellular alterations that do not progress, and those that are already progressing to something more serious, and therefore should be included in the computations, per EPA's guidelines, as discussed.

6) Elsewhere, the PWG report says: "The purpose of the PWG review was to determine the incidence of hepatic neoplasms in female rats following currently accepted nomenclature and diagnostic criteria (Goodman, et al., 1994) and to discuss the relevance, for purposes of risk assessment, of the hepatic neoplasms which occurred in the study." (p. 10) Actually, the latter of these two objectives is not a requirement of the PR Notice 94-5. The PR Notice applies only to diagnoses (i.e. re-diagnosis) of proliferative lesions. Under the PR Notice, such revised diagnoses are submitted to the Agency, <u>which in turn provides the assessment</u>. This is not to say the registrant is precluded from providing his own carcinogenicity assessment, but is that the duty, or something to be expected, of the PWG panel which is convened under PR Notice 94-5 for the purpose of diagnosing slides. However, to the extent the PWG elected to provide a carcinogenicity assessment, notably absent is any evaluation of the role of hepatocellular alteration in that assessment.

7) Did the lesion "hepatocellular alteration" have stricter diagnostic criteria than employed in the original study assessment. In fact, do the criteria under Goodman et al (1994) raise the bar for all three lesions, hepatocellular alterations, adenoma and carcinoma? The problem I have as a reviewing toxicologist is in understanding what has changed in terms of diagnostic criteria with Goodman et al. The definitions and criteria for diagnosing hepatocellular alteration, adenoma and carcinoma are reasonably clear, but I am unable to understand how employing these criteria would yield differing diagnoses

than previously.

For example, what was the basis, or criteria, Drs. Bolte and Busey employed in designating four lesions as carcinoma prior to the PWG? We accept that Dr. Busey employed the same criteria as the PWG performed the next day. It is imperative this be explained, and just how pervasive is the use of these newer criteria in the wider scientific community. In other words, if the playing field shifts, how are we to evaluate comparative results?

Goodman et al does not explain the shift, such as through the use of some before and after examples. There is some reason to suspect in reading Goodman et al, that perhaps criteria are such that "hepatocellular alteration" is a more relevant end point in the natural history of neoplasia under these criteria. For example, Goodman says: "The differential diagnosis between foci of cellular alteration and hepatocellular adenoma.....(is) more controversial." (p. 8), presumably, meaning [more controversial] than distinguishing the older term "neoplastic nodule" from adenoma. "The committee recommends that the term hepatocellular adenoma be used for benign hepatocellular neoplasms rather than the term neoplastic nodule." (p. 8)
Now under current criteria, foci of cellular alteration and hepatocellular adenomas, compression of the surrounding liver and loss of lobular architecture are the main features to consider. "Significant compression, disruption of the normal architecture of hepatic lobules and plates, and/or presence of cellular atypia distinguish benign hepatocellular neoplasms from foci of cellular alteration." (p. 8) Elsewhere it is written "Except for occasional cellular atypia, the cells of adenomas are comparable morphologically to those described for foci of cellular alteration. Cytologic features which may be present (presumably in both adenoma and foci of cellular alteration) include cytoplasmic vacuolation, nuclear atypia, an increased nuclear/cytoplasmic ratio, and an increased mitotic index." (p. 3)

From passages such as these, I am inclined to conclude that foci of cellular alteration as characterized in Goodman et al are virtually the same qualitatively as those of adenoma, only not as large or advanced. Goodman et al say: "The incidence, size and/or multiplicity of foci usually are increased, and the time to development usually is decreased by the administration of hepatocarcinogens. Moreover, foci generally precede the development of tumors, and they have been categorized as preneoplastic." (p. 7) The text goes on to say they may or may not reverse. Furthermore, it should be noted that if criteria have recently changed, raising to a higher order the definition of adenoma or carcinoma, this may imply that such terms as "hepatocellular alteration" may have greater significance than previously in the characterization of the neoplastic process, and something needs to be said along these lines.

In other words, it is not sufficient to simply place the recent publication by Goodman et al (1994) in the PWG package without saying something about how this publication may have changed the interpretive process. More specifically, what has changed with this publication that made such a big difference in the interpretation of this malathion study? This is particularly important in view of the fact that even Dr. Busey seems to have misunderstood

the criteria of interpretation as recently as one day prior to the PWG meeting on March 15. What I glean from this report is that there is a continuum in the natural history of liver neoplasia, foci > adenoma > carcinoma, and that under EPA's cancer Guidelines, foci would be interpreted, conservatively, in the interest of the public health, as a "key event" in the neoplastic process and used accordingly by these Guidelines.

Conclusions:

1) The PR Notice under which the PWG was performed provides a mechanism for the diagnosis or re-diagnosis of histopathology findings. It does not call for an interpretation of carcinogenicity. Interpretation is the responsibility of the Agency in consideration of the entire data base. In this particular PWG, an interpretation of carcinogenicity was rendered, which incorporated not only the revised or final tumor diagnoses, but other data from the original bioassay, including such data as mortality, body weights, organ weights, etc. All but the tumor diagnosis data should be ignored.

2) The claimed purpose of the PWG was to determine the incidence of hepatic neoplasms, and not nonneoplastic lesions. Thus although foci of hepatocellular alterations were examined for possible re-diagnosis as neoplasms, the incidences of these were not tabulated.

3) No satisfactory explanation has been forthcoming from the PWG to explain in a manner consistent with the performance of a reliable peer review performed March 14, the downgrading on March 15 of several tumorigenic diagnoses concurred in by the study pathologist and reviewing pathologist, where the latter individual employed the same criteria as that of the PWG.

4) In spite of questions posed by HED seeking explanations in morphologic terms of the basis for changed diagnoses, none has been provided.

5) Review of relevant publications indicate that with respect to epithelial hepatocellular tumor development in the Fischer rat, there is the progression of lesions: hepatocellular alteration > adenoma > carcinoma, referred to in Eustis et al (1990) as the "natural history of neoplasia", and that hepatocellular alterations must be included with adenomas and carcinomas in the assessment of tumorigenicity under EPA's Guidelines.

6) EPA's Cancer Assessment Guidelines provide for the consideration of incidences of preneoplastic lesions (as "key events") in conjunction with incidences of the respective neoplasms in the assessment of a neoplastic response. In consideration of the Agency's perspective on the subject, the absence of final tabulated incidences of hepatocellular alterations of moderate degree of severity or greater following this peer review is problematic in assessing the tumorigenic response under EPA's Guidelines. Nonetheless, when a logical estimate of incidences of hepatocellular alterations of moderate degree of

severity is considered in conjunction with adenomas, there is a positive response across all doses. To further substantiate this assessment, the PWG should be requested to submit a tabulation of incidences of hepatocellular alterations of moderate or greater degree of severity.

7) Insofar as the PWG does not provide incidences of hepatocellular alterations to be used as "key events" under EPA's Guidelines in evaluating the tumorigenic response, the PWG report should be discounted, and the original diagnoses be retained.

8) It is possible that the diagnoses of hepatocellular lesions under the contemporary criteria amount to a downward frame shift in incidences of hepatocellular alterations, adenomas and carcinomas versus that under perhaps older criteria, such that more attention may need to be directed to addressing incidences of hepatocellular alteration of moderate degree of severity in assessing the neoplastic response under EPA's Guidelines.

9) In his letter of July 24, 1997, Dr. Robert Maronpot recommended inclusion of a "qualified pathologist from academia" on the PWG panel. Since such a pathologist was not among those composing the present PWG, and since as is implied in Dr. Maronpot's letter, the independent perspective of such a pathologist would be very constructive, I must recommend, particularly in light of the remarkable revisions in diagnoses from the PWG, that an additional interpretation of the slides (i.e. another opinion) be obtained from pathologists from academia before the results of this PWG are accepted.

10) Concern is expressed as to the relevance of the historical control data (particularly that of the performing laboratory) given the remarkable changes of diagnoses of tumors in the current PWG employing more contemporary and possibly stricter criteria.

References

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Brian Dementi, Ph.D., D.A.B.T.
Senior Toxicologist/HED/OPP

Attachment(s) 1